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ERRATA

Page 2, line 14, for 'coresponding' read 'corresponding'
Page 95, line 15 from bottom, for 'II' read '11'
Page 97, line 25, for '*moschata* Duchesne' read '*pepo* var. *condensa* Bailey'
Page 127, line 6 from bottom, for '1 to 2.3' read '2.3 to 1'
Page 147, line 5, for 'dioxide' read 'monoxide'
Page 189, line 20, for 'the' read 'to'
Page 301, line 4, for 'Fig. 1 C' read 'Fig. 1 B'
Page 344, line 10, for 'days' read 'day'
Page 366, line 1, for 'trees' read 'tree'

FURTHER EXPERIMENTS ON THE EFFECT OF HALOGENATED ALIPHATIC COMPOUNDS ON THE RESPIRATION OF POTATO TUBERS[1]

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In connection with studies on the metabolism of potato tubers (*Solanum tuberosum* L.) treated with chemicals which break dormancy (3) it has been shown that ethylene chlorhydrin (CH_2OHCH_2Cl) has a marked stimulatory effect on respiration as measured by the carbon dioxide output (9, 13). When potato tubers are exposed to ethylene chlorhydrin vapor the respiratory rate doubles within 10 to 12 hours after the start of the exposure (10) and gradually rises to a maximum in about 50 hours at which time the treated tubers respire from three to six times as rapidly as the controls. The maximum effect is thus not obtained until a considerable time after the end of treatment since treatment periods of only 24 hours' duration were used. Subsequently the respiratory rate gradually falls until after seven or eight days it reaches a value approaching that of the control. At this time visible development of sprouts at the eyes becomes evident.

Ethylene bromohydrin (CH_2OHCH_2Br), ethyl mercaptan (C_2H_5SH), acetaldehyde (CH_3CHO), hydrogen cyanide (HCN), and hydrogen sulphide (H_2S) also bring about increases of several hundred per cent in the CO_2 production (8, 9). On the other hand ethyl alcohol when applied in amounts which have a favorable effect in ending the dormant period causes a pronounced diminution of the respiratory activity. The effect of a chemical on the respiration of the tubers is, therefore, not correlated with its dormancy-breaking activity.

Determination of the effect of the alkyl halides, some of which have been reported to act as dormancy-breaking agents (3, 6) has shown that these compounds are powerful stimulants of the respiratory activity (11). A study of their relative efficacy has brought out the interesting fact that the activity of the alkyl halides in increasing respiration is closely related to their chemical reactivity. Thus the iodides are more active than the bromides, which in turn are much more active than the chlorides. The normal compounds are considerably more efficacious than the corresponding secondary compounds. Large increases in the CO_2 output are also induced by the less active of these compounds provided relatively higher concentrations are used.

The results of experiments with a number of additional compounds with emphasis on compounds closely related to ethylene chlorhydrin are re-

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 83.

ported in the present paper. Many of the chemicals have been investigated over a wide range of concentrations. The chemicals studied have included alkylene halides, alkylidene halides, trimethylene halides, halohydrins, and some additional chloro and bromo derivatives of ethane and ethylene. Tests with about 30 compounds are reported. All of these chemicals which are sufficiently volatile to be applied in the form of a vapor treatment are active in increasing the CO_2 output of potato tubers. Differences are evident in the concentrations required to cause large increases. The results indicate that, in so far as information is available, the chemical reactivity of these compounds is an important factor in connection with their effect on respiration. Thus, the bromides are more active than the chlorides, while the chlorobromides are intermediate in their effect. The alkylene compounds have a greater action than the alkylidene derivatives. The chlorohydrins and bromohydrins are less active than the coresponding dichlorides and dibromides but are also much less toxic. Of the compounds comprising the homologous series of alkylene bromides from methylene through butylene bromide, ethylene bromide is by far the most active. The efficacy of the butylene compounds is in the order α is greater than β is greater than *iso*. Trimethylene bromide is more effective than the isomeric propylene bromide. Ethylene bromide and chloride are more active than *s*-dichloro and *s*-dibromo ethylene.

Interesting differences have also been observed with regard to the range of effective concentration, i.e., the range from the lowest concentration which will cause an increased CO_2 output of at least 30 per cent under the conditions of these experiments to the highest concentration which can be tolerated without injury. This range of concentration was found to be very large for ethylene chlorhydrin and ethylene bromohydrin extending from 0.031 to 8.0 and from 0.016 to 4.0 millimols per liter respectively, while the similar range for the more active ethylene bromide extended only from 0.0045 to 0.036 millimol per liter.

MATERIALS AND METHODS

Tubers used. The potato tubers used for these experiments were obtained from a number of sources. Most of the tests were made on tubers grown in Bermuda or Florida (probably Bliss Triumph variety) and purchased in the Yonkers market. Some experiments were also conducted with tubers of the Irish Cobbler variety obtained from New Jersey in the fall of 1933 and in the spring of 1934 from South Carolina. The various lots of tubers did not show any qualitative differences in the responses caused by the chemicals but quantitative comparisons cannot be made between treatments made on tubers from different lots. The potatoes from Bermuda and Florida although purchased as new potatoes were no longer very dormant but as has been found from previous experiments

such tubers still respond to treatments with chemicals. The experiments of the present paper as well as those previously conducted have shown that as tubers age they gradually become somewhat less responsive to stimulation of the respiration, but no sharp break is evident as the dormant period ends.

In the tables given in the paper, individual experiments consisting of a number of treatments and a control are separated from each other by horizontal lines and the source of the tubers is indicated in those cases in which this information is necessary for a proper interpretation of the tables.

Chemicals used. The chemicals used in these tests (except ethane [CH_3CH_3] and ethyl alcohol) were obtained from the Eastman Kodak Company and were of the grade listed in their catalog as of the highest purity. The following chemicals were, however, further purified by redistillation before use, the first and last fractions being discarded: ethylene chlorhydrin, ethylene bromohydrin (in vacuo), ethylene chloride (CH_2Cl-CH_2Cl), ethylene chlorobromide (CH_2ClCH_2Br), ethylene bromide (CH_2BrCH_2Br), trichloroethane ($CHClCHCl_2$), ethyl bromide (C_2H_5Br).

Methods. The methods used were the same as those described in previous publications (8, 9). The tubers (750- or 500-gram samples were used depending upon the size of the tubers) were placed in 3-liter desiccators and exposed to the vapors of a definite amount of the chemical evaporating from cotton placed within the containers. After a treatment period of 24 hours a stream of CO_2-free air was drawn through the containers and subsequently through Van Slyke-Cullen tubes (16) containing standardized solutions of $Ba(OH)_2$. The tubes containing precipitated $BaCO_3$ were removed after two hours and the CO_2 given off during the treatment period and for two hours thereafter determined. The next period covered the 22 hours from 26 hours after the start of the treatment to 48 hours. From the data thus obtained the number of milligrams of CO_2 given off per 100 g. of fresh tissue in 48 hours was calculated and the increase over the control noted. These values were used for comparisons between the effects produced by various chemicals. The desiccators and the tubes were suspended in a thermostatically controlled water bath. All experiments were conducted at a temperature of 26° C.

In order to avoid a possible error from some unabsorbed halide reacting with the $Ba(OH)_2$ and being erroneously computed as CO_2 the air leaving the desiccators was first drawn through two Van Slyke-Cullen tubes containing 25 cc. of 10 per cent silver nitrate solution before being drawn through the $Ba(OH)_2$ solutions. As a further precaution the $Ba(OH)_2$ solutions were tested for halides after the absorption of the CO_2 and if any were present the amount was determined and the proper correction made (10). These corrections when necessary were small.

After the respiration determinations were ended the tubers were cut into pieces with one eye each and planted in flats. Observations were made on their growth to determine which treatments had produced injury.

TABLE I

EFFECT OF SOME RELATED CHEMICALS ON THE RESPIRATION OF POTATO TUBERS

Chemical	Concn., millimols per l.	Mg. CO_2 per 100 g. per hour		Total CO_2 per 100 g. in 48 hrs.	Increase over control
		0–26 hrs.	26–48 hrs.		
CH_2OHCH_2Cl	4	2.42	4.80	163.5	84.1
CH_2OHCH_2Br	4	3.14	6.10	215.8	136.4
Control	—	1.37	1.98	79.4	—
CH_2ClCH_2Cl	4	2.72	7.06	227.8	149.4
CH_2ClCH_2Br	4*	3.56	7.50	257.5	179.1
CH_2BrCH_2Br	4*	3.58	7.80	264.7	186.3
CH_3CHBr_2	4	3.39	6.62	233.6	155.2
Control	—	1.41	1.89	78.4	—
CH_3CH_2OH	2	1.99	2.44	105.2	0.5
CH_2OHCH_2OH	2	1.94	2.50	103.5	−1.2
CH_2OHCH_2Cl	2	2.50	6.07	198.1	93.4
CH_2OHCH_2Br	2	3.34	8.00	263.0	158.3
CH_2ClCH_2Cl	2	2.74	6.60	230.8	126.1
CH_2ClCH_2Br	2*	3.95	7.60	270.5	165.8
CH_2BrCH_2Br	2*	4.01	7.88	277.0	172.3
CH_3CHBr_2	2	3.88	6.06	234.4	129.7
Control	—	1.82	2.62	104.7	—
CH_3CH_2OH	1	3.26	3.63	164.7	29.1
CH_2OHCH_2OH	1	3.34	3.35	160.4	24.8
CH_2OHCH_2Cl	1	3.06	5.32	196.5	60.9
CH_2OHCH_2Br	1	3.61	6.97	247.3	111.7
CH_2ClCH_2Cl	1	3.37	5.63	211.7	76.1
CH_2ClCH_2Br	1*	5.10	7.22	291.5	155.9
CH_2BrCH_2Br	1*	4.80	7.57	291.0	155.4
CH_3CHBr_2	1	3.74	4.46	195.2	59.6
Control	—	2.70	2.98	135.6	—
CH_3CH_2OH**	8	0.58	1.32	44.1	—15.9
CH_2OHCH_2OH	8	1.28	1.56	67.6	7.6
CH_2OHCH_2Cl	8	2.32	3.44	136.3	76.3
CH_2OHCH_2Br	8*	2.48	4.82	165.1	105.1
CH_2ClCH_2Cl	0.5	1.85	3.90	134.0	74.0
CH_2BrCH_2Br	0.5*	2.34	4.51	160.5	100.5
CH_3CH_3	1.0	1.76	2.41	98.8	38.8
Control	—	1.10	1.42	60.0	—
CH_3CH_2OH	0.25	1.14	1.42	60.9	3.5
CH_2OHCH_2OH	0.25	1.27	1.35	62.8	5.4
CH_2OHCH_2Cl	0.25	1.86	3.29	120.8	63.4
CH_2OHCH_2Br	0.25	1.86	3.19	118.6	61.2
CH_2ClCH_2Cl	0.25	1.84	3.34	121.2	63.8
CH_2ClCH_2Br	0.25*	2.10	3.90	140.4	83.0
CH_2BrCH_2Br	0.25*	2.00	3.90	137.7	80.3
CH_3CHBr_2	0.25	1.20	1.47	68.2	11.5
CH_3CH_2Br	0.25	1.38	2.56	92.3	34.9
Control	—	1.04	1.38	57.4	—

TABLE I (*Continued*)

Chemical	Concn., millimols per l.	Mg. CO_2 per 100 g. per hour		Total CO_2 per 100 g. in 48 hrs.	Increase over control
		0–26 hrs.	26–48 hrs.		
CH_3CH_2OH	0.063	0.96	1.15	50.3	−3.5
CH_2OHCH_2OH	0.063	0.90	1.14	48.4	−5.4
CH_2OHCH_2Cl	0.063	1.21	2.59	88.5	34.7
CH_2OHCH_2Br	0.063	1.32	1.86	75.3	21.5
CH_2ClCH_2Cl	0.063	1.28	3.14	101.4	47.6
CH_2ClCH_2Br	0.063	1.77	3.05	113.2	59.4
CH_2BrCH_2Br	0.063*	2.78	3.44	148.1	94.3
CH_3CHBr_2	0.063	0.96	1.20	51.4	−2.4
CH_3CH_2Br	0.063	0.92	1.23	51.0	−2.8
Control	—	1.05	1.21	53.8	—
CH_2OHCH_2Cl†	0.031	1.56	2.44	94.2	28.9
CH_2OHCH_2Br	0.031	2.27	3.32	132.0	66.7
CH_2ClCH_2Cl	0.031	2.18	2.18	80.4	15.1
CH_2ClCH_2Br	0.031	2.48	3.36	138.6	73.3
CH_2BrCH_2Br	0.031	2.83	4.54	173.2	107.9
Control	—	1.18	1.58	65.3	—
CH_2OHCH_2Cl	0.016	1.45	2.10	83.6	9.1
CH_2OHCH_2Br	0.016	1.72	2.57	101.3	26.8
CH_2ClCH_2Cl	0.016	1.49	2.14	85.7	11.1
CH_2ClCH_2Br	0.016	2.76	3.14	141.0	66.5
CH_2BrCH_2Br	0.016	3.95	4.90	211.0	136.5
Control	—	1.23	1.92	74.5	—
CH_2OHCH_2Cl	0.008	1.34	2.42	88.2	11.3
CH_2OHCH_2Br	0.008	1.28	2.72	93.4	16.5
CH_2ClCH_2Cl	0.008	1.35	1.91	77.1	0.2
CH_2ClCH_2Br	0.008	1.99	2.96	116.9	40.0
CH_2BrCH_2Br	0.008	2.24	3.42	133.8	56.9
Control	—	1.20	2.06	76.9	—

* These treatments produced severe injury.

** The experiments in this and the two following sections of the table were carried out with potatoes obtained from Florida. For the first four series potatoes from Bermuda were used.

† Freshly-harvested Irish Cobbler potatoes were used for the last three series listed. Very dilute aqueous solutions of the chemicals were prepared in order to be able to measure out the small quantities required to obtain concentrations of 0.016 and 0.008 millimol per liter.

All treatments which injured the tubers to the extent that 50 or more per cent failed to germinate are designated in the tables as having produced severe injury.

RESULTS

WITH COMPOUNDS RELATED TO ETHYLENE CHLORHYDRIN

The effects of various concentrations of a number of related chemicals on potato tuber respiration are tabulated in Tables I and II. The chemicals concerned may be considered as mono- and di-substitution products of ethane, in which one or two hydrogens have been replaced by a chlorine or bromine atom or the OH group or by combinations of these substituents.

TABLE II

EFFECT OF TREATMENTS WITH ETHYLENE BROMIDE ON THE RESPIRATION OF IRISH COBBLER POTATO TUBERS

Concn., millimols per l., 24 hrs.	Mg. CO_2 per 100 g. per hour at intervals after start of treatment		Mg. CO_2 per 100 g. in 48 hrs.	Increase over control
	0–26 hrs.	26–48 hrs.		
0.58*	2.62	7.23	227.2	184.5
0.29*	2.41	5.82	191.1	148.4
0.145*	2.45	5.05	175.0	132.3
0.072*	2.09	4.46	152.5	109.8
0.036*	2.08	3.64	132.0	89.3
Control	0.74	1.06	42.7	—
0.072*	2.32	4.32	155.5	114.8
0.036	2.13	3.48	126.9	86.2
0.018	1.52	2.66	98.1	57.4
0.009	1.15	2.18	77.8	37.1
0.0045	0.90	1.36	53.4	12.7
0.0022	0.69	1.08	41.5	0.8
0.0011	0.62	1.01	38.3	−2.4
Control	0.69	1.03	40.7	—

* These treatments produced severe injury.

Many of the chemicals have been tested over a series of concentrations covering the range from the lowest concentration which has an appreciable action to the concentration which results in injury to the tubers. This range of effective concentrations for the chemicals studied is shown in Table III.

In Table I individual experiments consisting of tests with a number of chemicals and a control are separated from each other by horizontal lines. Not all of the experiments were made on tubers from the same lot so that quantitative comparisons between them cannot be made but the relative efficacy of the various chemicals studied holds quite well throughout and

TABLE III

EFFECTIVENESS OF SOME RELATED CHEMICALS IN INCREASING RESPIRATION

Order of effectiveness	Approx. effective range.* Millimols per l.	Ratio $\frac{\text{Highest concn.}}{\text{Lowest concn.}}$
CH_2BrCH_2Br	0.0045–0.036	8
CH_2ClCH_2Br	0.0045–0.063	14
CH_2OHCH_2Br	0.016 –4.0	250
CH_2ClCH_2Cl	0.063 –4.0	64
CH_3CHBr_2	0.25 –4.0	16
CH_2OHCH_2Cl	0.031 –8.0	250
CH_2OHCH_2OH	—	—

* The effective range is here defined as the range from the lowest concentration which will increase the CO_2 output under the conditions of these experiments at least 30 per cent to the maximum concentration which can be tolerated by the tubers without injury.

the conclusions reached in this regard are thus further fortified by the fact that tubers from different sources gave the same results.

The data show that ethylene bromide (CH_2BrCH_2Br) and ethylene chlorobromide (CH_2ClCH_2Br) are the most effective in increasing respiration at all the concentrations tried (from 0.008 to 4.0 millimols per liter), the dibromide being slightly more efficacious than the chlorobromide. However, these compounds are also very toxic, as has been previously observed by Denny for ethylene bromide (3), and at most of the concentrations used the treatments were lethal. The results with ethylene bromohydrin show that when an OH group is substituted for one of the bromine atoms the new compound does not have a very greatly lessened activity as a stimulant of the respiration but has a much lower toxicity. The value obtained by dividing the maximum concentration which can be tolerated by the tubers without injury by the lowest concentration which will still cause an increased CO_2 production of about 30 per cent is 250. A ratio derived in the same way for the other chemicals is much smaller except for ethylene chlorhydrin which has an effective range of about equal magnitude. The effect of substituting an OH group for a halogen in lessening toxicity without so markedly lessening the stimulatory action on respiration is also evident in comparisons between ethylene chloride and ethylene chlorhydrin. When an OH group is substituted for both of the halogens the resultant compound (CH_2OHCH_2OH) is found to have very little effect.

The increased efficacy of ethylene bromide and chlorobromide over the other chemicals is clear cut and evident at all concentrations. Some of the chemicals, however, show different relative efficacies at different concentrations. This is due to the fact that the form of the curve obtained when the concentration is plotted against the increase in CO_2 produced is not identical for all the chemicals. Thus ethylene chloride and ethylidene bromide (CH_3CHBr_2) are quite active stimulants at high concentrations and exceed ethylene chlorhydrin in their effect at 4 and 2 millimols per liter but as the concentrations are decreased the curves for ethylene chloride and ethylidene bromide fall more rapidly than that of ethylene chlorhydrin. Thus ethylene chlorhydrin still produces a 30 per cent increase at a concentration of 0.031 millimol per liter, while ethylidene bromide ceases to have any effect at 0.063 millimol per liter and ethylene chloride is less effective than ethylene chlorhydrin at low concentrations.

The chemicals considered may be arranged in the following order of effectiveness in increasing respiration, the most effective being listed first: ethylene bromide, ethylene chlorobromide, ethylene bromohydrin, ethylene chloride and ethylidene bromide, ethylene chlorhydrin, ethylene glycol, ethyl alcohol. Ethylene chloride and ethylidene bromide fall below ethylene chlorhydrin at low concentrations. Ethyl bromide is less active than

the dibromide and the bromohydrin at the concentrations studied. Ethylene glycol (CH_2OHCH_2OH) has little effect at any concentration. Ethyl alcohol differs from the halogen derivatives in that it depresses the respiration at high concentrations (9, 10). In the present experiments treatments with 1 millimol showed slight stimulation, with 2 and with 0.25 millimols no change was produced, while 8 millimols caused a depression. From previous experiments it is known that higher concentrations cause further depressions. The lethal concentration is about 65 millimols.

The data of Table I show that at a concentration as low as 0.008 millimol per liter, ethylene bromide and ethylene chlorobromide still increase the CO_2 output over 50 per cent. The lower limit of effective treatment for ethylene bromide was determined in another experiment. The data are given in Table II. A concentration of 0.0045 millimol produced an increase of about 30 per cent, while concentrations of 0.0022 and 0.0011 millimol had no effect. The lethal concentration was found to be 0.036 or 0.072 millimol. These results fit in well with those of Table I although obtained on tubers from a different lot. It is of interest to note that the higher the concentration the greater the increased CO_2 output even though the concentrations were increased up to 8 or 16 times the minimum lethal treatment. Large increases have also been found to occur with concentrations 60 times the lethal concentration (Table I).

WITH ALKYLENE BROMIDES AND RELATED COMPOUNDS

Further data on the relative efficacies of various alkylene and alkylidene halides are given in Table IV. The results show that of the compounds comprising the homologous series, methylene bromide (CH_2Br_2), ethylene bromide (CH_2BrCH_2Br), propylene bromide ($CH_3CHBrCH_2Br$), and butylene bromide ($C_4H_8Br_2$), the second member, ethylene bromide, is by far the most active in increasing the CO_2 output. The other members of the series also cause large increases, however. Of the three isomeric butylene bromides studied, the α compound ($CH_3CH_2CHBrCH_2Br$) is more effective than the β compound ($CH_3CHBrCHBrCH_3$) which in turn has a more powerful action than *iso*-butylene bromide [$(CH_3)_2CBrCH_2Br$]. From the viewpoint of a possible relation between toxicity and the effect on the CO_2 output it is of interest to note that although α butylene bromide is more effective in increasing respiration it is less toxic than the β compound.

Trimethylene bromide ($CH_2BrCH_3CH_2Br$) produces a larger increase than the same concentration of the isomeric propylene bromide. The table also gives further data on the greater efficacy of ethylene bromide compared with the isomeric ethylidene bromide, and similarly ethylene chloride is found to be more effective than ethylidene chloride.

TABLE IV

EFFECT OF SOME ALKYLENE BROMIDES AND RELATED COMPOUNDS ON THE RESPIRATION OF POTATO TUBERS

Chemical	Concn. millimols per l.	Mg. CO_2 per 100 g. per hour		Total CO_2 per 100 g. in 48 hrs.	Increase over control
		0–26 hrs.	26–48 hrs.		
CH_2BrCH_2Br	0.5*	1.59	3.45	117.2	73.5
$CH_3CH_2CHBrCH_2Br$	2.0*	1.52	2.88	103.0	59.3
$CH_3CHBrCHBrCH_3$	2.0*	1.38	2.57	92.5	48.8
Control	—	0.82	1.02	43.7	—
CH_2BrCH_2Br	1.0*	1.52	3.12	107.9	57.5
$CH_3CHBrCH_2Br$	1.0*	1.31	2.38	86.3	35.9
$CH_2BrCH_2CH_2Br$	1.0	1.50	2.48	93.5	43.1
$CH_3CH_2CHBrCH_2Br$	1.0	1.57	2 65	99.0	48.6
$CH_3CHBrCHBrCH_3$	1.0*	1.40	2.22	85.4	35.0
Control	—	0.93	1.19	50.4	—
CH_2Br_2	4.0*	1.48	3.44	114.1	71.7
CH_2ClCH_2Cl	4.0	1.20	2.59	88.2	45.8
CH_3CHCl_2	4.0	1.15	2.00	73.9	31.5
CH_2BrCH_2Br	0.5*	1.54	3.24	111.1	68.7
CH_3CHBr_2	4.0*	1.09	2.04	73.4	31.0
$CH_3CH_2CHCl_2$	4.0	1.12	1.96	72.2	29.8
$(CH_3)_2CBrCH_2Br$	4.0	1.09	1.79	67.8	25.4
Control	—	0.77	1.02	42.4	—
CH_2BrCH_2Br	2.0*	1.62	3.17	111.8	62.4
CH_3CHBr_2	2.0	1.45	2.47	92.1	42.7
CH_3CHCl_2	2.0	1.00	1.85	67.3	17.9
$CH_3CHBrCH_2Br$	2.0*	1.39	2.34	87.6	38.2
$CH_3CH_2CHCl_2$	2.0	1.46	2.31	88.8	39.4
$CH_3CH_2CHBrCH_2Br$	2.0	1.64	3.11	111.1	61.7
$CH_3CHBrCHBrCH_3$	2.0*	1.53	2.57	96.3	46.9
$(CH_3)_2CBrCH_2Br$	2.0	1.11	1.85	69.5	20.1
Control	—	0.89	1.15	49.4	—
CH_2Br_2	0.5	1.54	2.37	92.0	49.8
CH_2BrCH_2Br	0.5*	1.72	3.56	123.0	81.8
CH_3CHBr_2	0.5	1.15	1.96	73.3	31.1
$CH_3CHBrCH_2Br$	0.5	1.18	2.26	80.4	38.2
$CH_2BrCH_2CH_2Br$	0.5	1.49	2.86	102.6	60.4
$CH_3CH_2CHBrCH_2Br$	0.5	1.14	2.31	80.4	38.2
$CH_3CHBrCHBrCH_3$	0.5	1.20	2.23	80.2	38.0
$(CH_3)_2CBrCH_2Br$	0.5	1.04	2.09	70.3	28.1
Control	—	0.75	1.04	42.2	—

* These treatments produced severe injury.

WITH TRIMETHYLENE COMPOUNDS

The effect of treatments with 2 and 1 millimols per liter of some related trimethylene compounds is shown in Table V. In general the same relations hold as were found for the corresponding ethylene compounds except that the bromohydrin ($CH_2OHCH_2CH_2Br$) was found to be much less effective than one would expect. This is in all probability due to the high boiling point (62° C. at 5 mm. pressure) of this compound, since the method

used in these experiments demands that the chemicals applied to cotton within the container be rather readily vaporized. The same reason also probably accounts for the relatively slight action of the tribromo compound ($CH_2BrCHBrCH_2Br$) with a boiling point of 220° C.

TABLE V

EFFECT OF SOME TRIMETHYLENE COMPOUNDS ON THE RESPIRATION OF POTATO TUBERS

Chemical	Concn. millimols per l.	Mg. CO_2 per 100 g. per hour		Total CO_2 per 100 g. in 48 hrs.	Increase over control
		0–26 hrs.	26–48 hrs.		
$CH_2OHCH_2CH_2Cl$	2.0	1.04	2.17	74.7	34.3
$CH_2OHCH_2CH_2Br$	2.0	1.11	1.12	53.4	13.0
$CH_2ClCH_2CH_2Br$	2.0	1.49	2.71	98.2	57.8
$CH_2BrCH_2CH_2Br$	2.0	1.43	2.78	97.2	56.8
$CH_2BrCHBrCH_2Br$	2.0	0.97	1.46	57.4	17.0
CH_2OHCH_2Cl	2.0	1.15	2.11	76.5	36.1
CH_2OHCH_2Br	2.0	1.35	2.65	93.5	53.1
Control	—	0.72	0.98	40.4	—
$CH_2OHCH_2CH_2Cl$	1.0	1.15	2.26	79.7	33.8
$CH_2OHCH_2CH_2Br$	1.0	1.15	1.70	67.4	21.5
$CH_2ClCH_2CH_2Br$	1.0	1.54	2.59	97.0	51.1
$CH_2BrCH_2CH_2Br$	1.0	1.52	2.78	100.8	54.9
$CH_2BrCHBrCH_2Br$	1.0	0.93	1.29	52.7	6.8
CH_2OHCH_2Br	1.0	1.31	2.39	86.7	40.8
Control	—	0.83	1.11	45.9	—

WITH CHLORO AND BROMO DERIVATIVES OF ETHANE AND EHTYLENE

The effectiveness of a number of di- and trichloro and bromo derivatives of ethane and ethylene is shown in Table VI. Ethylene bromide (CH_2BrCH_2Br) was found to cause larger increases in respiration than any other derivative studied. Dibromoethylene ($CHBr:CHBr$) and dichloroethylene ($CHCl:CHCl$) are considerably less effective than the corresponding saturated compounds at all the concentrations tested. The definite superiority of trichloroethane over tribromoethane is contrary to what was previously found in regard to the relative efficacy of chloro and bromo compounds and is probably due to the comparative non-volatility of the tribromo derivative (B. P. 181° C.; for trichloroethane, 113° C.).

EFFECT OF THE TREATMENTS ON THE PH OF THE EXPRESSED JUICES

In each case at the end of the 48 hours during which the respiration was determined the tubers were cut into pieces containing one eye each and planted. Those portions which were not planted were passed through a nixtamal mill and the juice pressed out through cheesecloth. Determinations were made of the pH values of the juices with the use of the quinhydrone electrode. All treatments which increased the respiration also increased the pH of the expressed juice. Increases varied from slight

changes in cases in which the respiration increases were small to increases of about 0.5 pH.

TABLE VI

EFFECT OF SOME CHLORO- AND BROMO-DERIVATIVES OF ETHANE AND ETHYLENE ON THE RESPIRATION OF POTATO TUBERS

Chemical	Concn. millimols per l.	Mg. CO_2 per 100 g. per hour		Total CO_2 per 100 g. in 48 hrs.	Increase over control
		0–26 hrs.	26–48 hrs.		
CH_2BrCH_2Br	1.0*	1.52	3.12	107.9	57.5
$CH_2ClCHCl_2$	1.0*	1.52	2.74	100.0	49.6
$CH_2BrCHBr_2$	1.0	1.33	2.37	86.7	36.3
$CHBr{:}CHBr$	1.0	1.27	2.25	82.5	32.1
$CHBr{:}CBr_2$	1.0*	1.51	1.96	82.4	32.0
Control	—	0.93	1.19	50.4	—
CH_2BrCH_2Br	0.5*	1.59	3.45	117.2	73.5
$CH_2ClCHCl_2$	2.0*	1.39	2.51	91.4	47.7
$CH_2ClCHCl_2$	0.5	1.58	2.76	101.8	58.1
$CHBrCHBr_2$	2.0	1.21	2.34	83.0	39.3
$CHBrCHBr_2$	0.5	1.08	1.92	70.4	26.7
$CHBr{:}CHBr$	2.0*	1.28	2.53	88.8	45.1
$CHBr{:}CHBr$	0.5	1.35	2.01	79.4	35.7
Control	—	0.82	1.02	43.7	—
CH_2ClCH_2Cl	2.0	1.35	2.82	97.2	46.6
$CH_2ClCHCl_2$	2.0*	1.28	2.84	95.9	45.3
$CH_2BrCHBr_2$	2.0	1.35	2.42	88.4	37.8
$CHBr_2CHBr_2$	2.0	0.90	1.41	54.4	3.8
$CHCl{:}CHCl$	2.0	0.86	1.47	54.6	4.0
$CHBr{:}CHBr$	2.0*	1.39	2.56	92.4	41.8
$CHBr{:}CBr_2$	2.0*	1.44	1.95	85.1	34.5
Control	—	0.89	1.24	50.6	—

* These treatments produced severe injury.

RELATION BETWEEN PROPERTIES OF CHEMICALS AND THEIR EFFECTIVENESS IN INCREASING RESPIRATION

In these experiments in which the tubers are treated by placing them in a closed container with a definite amount of the chemical placed on cotton within the container, it is necessary that the chemicals vaporize readily and permeate the tubers in a comparable manner. The various chemicals studied have markedly different boiling points and it is also unlikely that the rates of penetration and diffusion within the tubers are the same for the different chemicals. In spite of these difficulties inherent in such a study of physiological reactions the experiments with the alkyl halides indicated that the effectiveness of these compounds was closely related to their chemical reactivity (11). In the case of the chemicals studied in the present paper data for chemical reactivity are in many instances not available. The greater activity of bromo compounds in increasing respiration as compared with chloro derivatives is evident except in a few in-

stances in which the bromo compounds have a very high boiling point, and consequently a low vapor pressure. Also the generalizations made by Tronov and Laduigina (15) as a result of their study of the activity of the halogen in some aliphatic polyhalogen compounds agree quite well with the activity of these compounds in their effect on respiration.

In view, however, of the limited data available and the uncontrollable factors in the method of testing these compounds it would be premature to conclude that the chemical reactivity is the most important property of these compounds upon which their effectiveness in increasing respiration depends.

DISCUSSION

The data presented in the first part of the paper show that when equimolecular concentrations are compared some of the chemicals are superior to ethylene chlorhydrin in their ability to increase the CO_2 production of potato tubers. For practical purposes, however, if one desires to increase the respiration as much as possible without producing injury, the lesser toxicity of ethylene chlorhydrin becomes important since a relatively higher concentration of the chlorhydrin can be used. Thus if we compare the efficacy of a safe concentration of chlorhydrin with a safe concentration of the other chemicals it is quite likely that the increased respiration resulting from the chlorhydrin treatment will be as great or greater than that of the other chemicals. Also the experiments in this paper report the effect of the treatments for the first 48 hours only. This time period was chosen because previous experiments had indicated that the maximum effect of the treatments was reached very close to 48 hours after the start of the treatment. It is possible that the chemicals might also show differences with regard to the length of time the stimulating effect continues after the end of the treatment. Some unpublished experiments in which the determinations of CO_2 output have been continued for longer periods indicate that the stimulatory action of ethylene chlorhydrin is more prolonged than that of ethylene chloride and butyl bromide. This is understandable in view of the much greater solubility of ethylene chlorhydrin in water, which might very possibly lead to a greater absorption of this chemical as compared with chemicals of lesser solubility. Preliminary experiments (unpublished) have shown that at least some of the absorbed ethylene chlorhydrin is again given off unaltered (or at least in such a form as to react with barium hydroxide to form barium chloride) and that it continues to be given off for a number of days after treatment. With all these facts in mind ethylene chlorhydrin can still be considered as one of the most efficacious, if not the most efficacious, in increasing respiration of all the chemicals thus far investigated.

The chemicals tested in this paper, as well as the alkyl bromides reported on previously, may all be considered as anaesthetics since according

to Sollmann (14, p. 750) the production of narcosis is a universal action of all members of the aliphatic series which are fat solvents and which are absorbable into the tissues. Considerable work has been done previously on the effect of anaesthetics on respiration. Some of this work is referred to in the textbooks of plant physiology. A somewhat more comprehensive review is given in Winterstein's book on narcosis (17). Practically all of these experiments have been made on plant material other than potato tubers except those of Appleman (1) who has previously found that ethyl bromide increases the respiration of potato tubers. Most of the workers have reported that anaesthetics cause an increase in respiration followed by a decrease below normal. Some, as for example Irving (5) and Denny (2), have found that with low concentrations an increase can result without a subsequent decrease below normal. Smith (12) who has studied the effect of alcohol, ether, and chloroform on the respiration of wheat, rye, and oat seedlings found that these chemicals first effect a decrease, followed by an increase and a final depression. Some experiments conducted by her on the effect of anaesthetics upon the permeability of the plasma membrane of *Ipomoea Learii* to CO_2 showed that the effect is a decrease followed by an increase. In view of this result she raises the point that the true effect of anaesthetics on plant respiration may be a profound depression, and that the apparent outburst of carbon dioxide at the maximum may simply be the release of CO_2 retained in the cell during the period of decreased permeability and not an increase in respiration.

Although the experiments of Guthrie (4) have shown that ethylene chlorhydrin increases the permeability of potato tubers as measured by changes in electrical conductivity and increased leaching of electrolytes from tissue suspended in distilled water, the increased CO_2 given off by treated tubers is very definitely not merely due to increased permeability but to an actual increase in the CO_2 production. This conclusion is reached as a result of the following considerations. If the increased CO_2 output were due to increased permeability and consequent outburst of CO_2 previously held within the tubers one would expect the permeability of the treated tubers finally to return to normal sometime after treatment. This would result in a CO_2 output below normal. Such a decrease below normal has not been observed even though measurements of the respiration have been continued for a week or more after the treatment (9). Furthermore the total increase in the CO_2 produced is so large that it is not probable that such a large amount of CO_2 could have been retained in the tubers previous to the beginning of the experiment. Also if the increased CO_2 production of treated tubers were due to an outburst of previously formed CO_2 then the treated tubers should, some hours after treatment, contain less CO_2 than the control tubers, the permeability of which has remained

unaltered. That such is not the case is indicated by previous experiments in which the CO_2 content of the expressed juices has been found to be higher in the case of treated than in control tubers (7). However, to eliminate all doubt on this point the CO_2 contained in treated and control tubers has been determined (unpublished) by immersing the tubers in boiling water and determining the CO_2 given off. The results have shown that the treated tubers contain more CO_2 than the control tubers, thus showing that the treated tubers not only give off more CO_2 than the controls but also contain more CO_2 and proving that there is an actual increase in CO_2 formation. An increased CO_2 content of the interior gas of ethylene chlorhydrin-treated tubers (dry) has also been shown by Smith (13, p. 286).

All of the 50 to 60 chemicals (8, 9, 11) which have been tested as to their effect on the CO_2 output of potato tubers may be divided into two classes: (1) Chemicals which cause a slight stimulation at low concentrations and a depression at higher concentrations and (2) chemicals which cause increases at all concentrations which have any effect, even if concentrations much above the minimum lethal concentration are used. To the first group belong ethyl, methyl, *n*-propyl, and *iso*-propyl alcohols, *n*- and *iso*-propyl ethers, and acetone; to the second, all the halogen derivatives which have thus far been studied, ethyl mercaptan, hydrogen sulphide, hydrogen cyanide, acetaldehyde (8, 9, 10, 11), and carbon disulphide (data unpublished).

When potato tubers are exposed to injurious concentrations of various chemicals, the injurious effect is made evident by the fact that the tubers decay. The organisms which cause decay no doubt also give off CO_2 and determinations of the CO_2 output after decay has set in are obviously worthless. Such injury is, however, not evident until some time after treatment and at the end of 48 hours, at which time the respiration determinations reported in this paper were terminated, the tubers usually appear quite sound even if they have been sufficiently injured to decay upon subsequent planting. In the case of chemicals of the second group no decrease in the CO_2 output has been observed at any concentration tested nor at any time after the treatment even when the respiration determinations were continued until active decay set in (when, of course, the CO_2 output is high partly because of the organisms associated with decay). In the case of chemicals of the first group high concentrations cause a marked diminution of the CO_2 output which becomes evident before the treatment is ended. After the end of the treatment period the respiration slowly rises to the normal level within a few days if recovery takes place. There is another difference, therefore, between the two groups of chemicals in that when treatments are made with chemicals of the first group (with concentrations which depress respiration) the maximum

effect of the chemical takes place during the period of treatment; after the end of treatment recovery to normal begins, or if very high concentrations have been used, decay sets in. The chemicals of the second group, however, do not exhibit their maximum effect until about 24 hours after the end of the treatment; the respiratory activity continues to rise for a considerable time after the tubers are removed from the influence of the vapor.

There may, therefore, be a fundamental difference in the mode of action of the two groups of chemicals on potato tuber respiration. However, when treatments are made with low concentrations which show slight stimulation (slight compared with the chemicals of the second group), the chemicals of the first group also show the maximum effect after the end of the treatment. This would indicate a similarity to the chemicals of the second group. In any event this difference is clear; when potato tubers are killed by exposure to the chemicals of one group marked diminution of the respiratory activity takes place before decay sets in; when killed by exposure to the vapors of chemicals of the second group marked stimulation takes place and no depression can be demonstrated before invasion by the organisms which cause decay.

SUMMARY

Experiments with about 50 halogenated derivatives of the aliphatic hydrocarbons [alkyl halides (11), alkylene halides, alkylidene halides, trimethylene halides, halohydrins, chloro and bromo derivatives of ethane and ethylene] have shown that all of these compounds which are sufficiently volatile are powerful stimulants of the respiratory activity of potato tubers when the tubers are exposed to the vapor in a closed container for a period of 24 hours. The effect of these chemicals is similar in that they all produce a very prompt rise in the respiratory activity soon after the beginning of the 24-hour treatment period which reaches a maximum only some time after the end of the treatment. Treatments which have any effect at all always result in increases in the CO_2 output; decreases have not been observed even when concentrations which were many times the minimum lethal concentration were used.

Differences in the action of these chemicals are evident, however, with regard to the concentrations necessary to cause large increases and in the range of concentrations from the smallest amount which will cause an increase to the highest concentration which can be tolerated without injury.

Thus when similar low concentrations are used the alkyl bromides are much more effective than the chlorides; the iodides somewhat more effective than the bromides. The normal halides are more active than the corresponding secondary compounds (11). Ethylene bromide is more active than ethylene chlorobromide which is in turn more effective than

ethylene chloride. The ethylidene compounds are less active than the ethylene compounds. Ethylene chlorhydrin and ethylene bromohydrin are less active than the corresponding dichloride and dibromide but are much less toxic. The effective ranges of concentrations (concentration producing at least a 30 per cent increase in CO_2 output to the highest concentration which can be tolerated without injury) for ethylene bromohydrin and ethylene chlorhydrin extend from 0.016 to 4.0 and 0.031 to 8.0 millimols per liter, respectively, and for ethylene bromide and ethylene chloride, from 0.0045 to 0.036 and from 0.063 to 4.0 millimols per liter. Of the compounds comprising the homologous series, methylene, ethylene, propylene, and butylene bromide, ethylene bromide is the most effective. Propylene bromide is less active than the isomeric trimethylene bromide; α butylene bromide is more active than the β compound which is in turn more active than *iso*-butylene bromide. Ethylene bromide and chloride are more efficacious than dibromo and dichloroethylene.

A comparison of the relative effectiveness of these compounds with their relative chemical reactivity indicates that the effect on respiration is closely identified with their chemical properties.

Treatments which increase the respiration also cause increases in the pH of the expressed juices.

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FACTORS INFLUENCING THE ACTIVITY OF TOBACCO MOSAIC VIRUS PREPARATIONS

A. W. PETRE

In the procedure for the purification of tobacco mosaic virus recommended by Vinson and Petre (9) a pigment-free preparation was obtained from juice of diseased plants by preliminary precipitation with basic lead acetate. In the supernatant liquid from this precipitate virus was thrown out by neutral lead acetate and from the latter precipitate pigment was removed by M/3 mono-potassium phosphate pH 4.5. From the pigment-free precipitate virus was removed by M/15 phosphate buffer pH 6.5.

The lead purification procedure, because of its ease of handling and rapid operation, lends itself readily to the preparation of purified virus. It has been used by Barton-Wright and McBain (1, 2) in a study of the chemical nature of the tobacco mosaic virus, also by Caldwell (3) and Stanley (8).

The following modifications of the original lead acetate precipitation method of Vinson and Petre (9) are suggested in the present paper on the basis of new relationships established between pigment, virus, and buffers: (a) An increase in the quantity of dilute acid phosphate buffer is without effect in eluting an increased concentration of pigment but a higher concentration of potassium acid phosphate is effective. (b) Increase in acidity of the potassium acid phosphate buffer used in the preliminary elution increases the activity of the virus removed in the final eluate but is less effective in removing pigment. (c) Following elution of pigment with higher concentrations of mono-potassium phosphate the lead acetate precipitates from field juice samples may require an alkaline phosphate buffer of pH higher than 6.5 to demonstrate their full activity.

The present experiments also show that the leaves of tobacco plants grown within glass cages are less susceptible to infection by virus, and it is suggested that this is due to the greater succulence of the cage-grown plants.

METHOD OF INOCULATION

The methods followed in this paper are those previously outlined by Vinson and Petre (9). Virus concentration measurements were made on Turkish tobacco plants (*Nicotiana tabacum* L.) grown in flats and inoculated by the method of Holmes (5), inoculating each plant by puncturing with five pins previously dipped in the virus solution. Where fewer than 150 plants were used the method of inoculation was modified by inoculating three leaves. Juice samples were obtained from frozen diseased Turkish tobacco plants pressed out with a hydraulic press. Horne's basic lead

TABLE I

EFFECT OF THE CONCENTRATION OF PHOSPHATE IN THE REMOVAL OF PIGMENT AND IN THE ELUTION OF VIRUS

Experiment No.	Concentration of phosphate: In the preliminary elution	Concentration of phosphate: In the elution to remove virus	Dilution of inoculum with ref. to juice	Fraction diseased
1	0.4, 0.5, 0.6 M KH_2PO_4	0.7 M mixed phosphate pH 6.7	1:3	1/10
	0.4, 0.5, 0.6 M KH_2PO_4	1.0 M mixed phosphate	1:3	5/10
	0.4, 0.5, 0.6 M KH_2PO_4	1.3 M mixed phosphate	1:3	2/10
2	0.4 M KH_2PO_4 0.4, 0.5, 0.6, 0.7 M KH_2PO_4	0.5 M KH_2PO_4 M/3 mixed PO_4 pH 6.7	2:3 2:3	1/10 1/10
	0.75 M KH_2PO_4 0.75, 0.8, 0.9, 1.00 M KH_2PO_4	0.75 M KH_2PO_4 M/3 mixed PO_4	1:2.5 2:3	3/10 1/10
	1.1 M KH_2PO_4 1.1, 1.2, 1.3, 1.3 M KH_2PO_4	1.2 M KH_2PO_4 M/3 mixed PO_4	1:3.5 2:3	3/10 2/10
	1.4 M KH_2PO_4 1.4, 1.5, 1.6, 1.7 M KH_2PO_4	1.5 M KH_2PO_4 M/3 mixed PO_4	1:4.5 2:3	3/10 2/10
3	M/3 acid PO_4 pH 3.5	Mixed PO_4 M/3	1:2	5/10
	M/3 acid PO_4 pH 4.0	Mixed PO_4 M/3	1:2	5/10
	Acid PO_4 1 M pH 3.5	Acid PO_4 1 M pH 3.5 Mixed PO_4 M/3	1:4 1:2	0/10 8/10
	Acid PO_4 1 M pH 4.0	Acid PO_4 1 M pH 4.0 Mixed PO_4 M/3	1:4 1:2	2/10 7/10
	Acid PO_4 1.7 M pH 3.5	Acid PO_4 1.7 M pH 3.5 Mixed PO_4 M/3	1:5 1:2	0/10 3/10
	Acid PO_4 1.7 M pH 4.0	Acid PO_4 1.7 M pH 4.0 Mixed PO_4 M/3	1:5 1:2	3/10 7/10
4	None	0.4 M KH_2PO_4	1:3	0/10
	None	0.7 M KH_2PO_4	1:3	3/10
	None	0.8 M KH_2PO_4	1:3	4/10
	None	1.0 M KH_2PO_4	1:3	1/10
5	M/3 KH_2PO_4	M/3 mixed PO_4 pH 6.7	2:3	3/10
	1.0 M KH_2PO_4	M/3 mixed PO_4	2:3	4/10
	1.0, 1.2 M KH_2PO_4	M/3 mixed PO_4	2:3	3/10
	1.0, 1.2, 1.3 M KH_2PO_4	M/3 mixed PO_4	2:3	1/10

acetate 200 g. per liter was used in clearing the juice in the proportions of 0.75 cc. to 25 cc. of juice. Neutral lead acetate 200 g. per liter was used to precipitate the virus from cleared juice samples in the proportions of 2 cc. to 25 cc. of juice.

Inoculations to detect traces of virus or to determine the effect of reagents on plants were made by wiping three leaves of each plant with a piece of cheesecloth wet with the solution.

PREPARATION OF PURIFIED VIRUS

Removal of pigment. The occurrence of greater concentrations of pigment along with higher concentrations of virus in field juice samples makes necessary some change in the conditions of the lead acetate method of purification in order to obtain a pigment-free final eluate. Successive elution of the precipitate at pH 4.5 with M/3 mono-potassium phosphate buffer removes very little additional pigment. The method may be adapted to the field juice samples by increasing the concentration of acid phosphate used in the preliminary elution and pigment-free eluates so obtained. As the concentration of the potassium acid phosphate buffer is increased some virus appears in this fraction. From Table I, experiments 2 and 4, around two-thirds molar is the concentration of the mono-potassium phosphate in which virus begins to appear. The pigment is removed at a concentration of M/1 so that under the best conditions attainable some virus appears in the acid phosphate eluate.

In Table I the neutral lead acetate precipitates were prepared by adding 2 cc. neutral lead acetate to 25 cc. field juice after preliminary clearing with 0.75 cc. basic lead acetate. Each horizontal division of the table represents a lead precipitate. In column 3 are given the eluates inoculated. On the same line in column 2 is given the previous treatment accorded the lead precipitate for the preparation of the eluate inoculated, and in column 4 is given the dilution of the inoculum with reference to the original juice.

Preliminary elution at pH 3.0. In Table II, although the phosphate eluate is more reactive prepared from the precipitate at pH 3.0, preliminary elution at lower pH is not so effective in removing pigment as the regular procedure.

Varying the reaction of the preliminary acid phosphate buffer has a noticeable effect on the activity of the virus preparation finally recovered. Within 0.4 to 0.6 of a unit the most favorable reaction for the acid phosphate buffer is pH 3.0. The higher pH serves better in removing the pigment; neutral phosphate eluates following elution at pH 3.0 with M/3 are more highly colored than those obtained following pH 4.5. Activity following preliminary elution at pH 3.0 is apparently at a maximum as compared with the pH values below 3.0. The lead precipitate appears to

be less soluble at pH 3.0 than at a pH below 3.0. At the lower pH values the preliminary eluate and subsequent water washes tend to be opalescent and some virus may be lost by dispersion of the precipitate at this point. In the experiments in Table II the lead precipitate prepared as in Table I was given two elutions with M/3 acid phosphate buffers, washed with water, and eluted finally with M/15 di-potassium phosphate pH 8.4.

TABLE II

PLANTS DISEASED OF TOTAL INOCULATED FOLLOWING PRELIMINARY ELUTION WITH POTASSIUM ACID PHOSPHATE BUFFERS OF DIFFERENT pH VALUES

pH	2.1	2.4	2.6	3.0	3.6	4.0	4.5
Exp. I	—	$\frac{6}{10}$	—	$\frac{8}{10}$	$\frac{2}{10}$	—	$\frac{4}{10}$
Exp. II	—	—	$\frac{26}{160}$	$\frac{73}{155}$	$\frac{23}{151}$	—	$\frac{37}{153}$
Exp. III	$\frac{37}{161}$	$\frac{66}{162}$	—	$\frac{76}{162}$	$\frac{57}{162}$	$\frac{55}{162}$	$\frac{31}{162}$

Optimum pH for elution of virus. The difference in behavior between the neutral and basic lead acetate precipitates noted (9) seemed to indicate activation and at the same time to infer a loss by the method of preparation followed in acid phosphate and neutral phosphate elution. The neutral lead precipitate could be prepared in a form that would yield suspensions by a preliminary elution with a solution of sodium arsenate adjusted to pH 4.5 by the addition of arsenic acid or arsenious acid and made to M/3 concentration or by substituting a more alkaline buffer for the 6.5 phosphate buffer. Following the elution with the above two reagents the lead precipitate yields water suspensions. However, the neutral lead acetate precipitate obtained in the form of a suspension could not be shown to be activated, because of the previous elution of the virus by the alkaline buffer. This 8.4 eluate when tested was found to be more active than eluates prepared at lower pH.

Plants treated directly with 8.4 phosphate and inoculated with juice to determine whether the effect of the higher reaction was on the virus or on the plant showed a decrease in the number of plants diseased. In this experiment the leaf to be inoculated with diseased juice was swabbed with a M/3 potassium phosphate buffer solution pH 8.5 using the edge of a glass slide to spread the solution across the surface of the leaf. Following two such treatments on successive days a dilute virus solution was introduced into the leaf by five pin punctures. Proceeding in this way no activation could be demonstrated so that it is not possible to attribute the effect of the alkaline phosphate buffer to its effect on the virus or its effect on the plant. In Table III the eluate at pH 8.4 is the most consistently

high reacting preparation. There are several factors involved and it is likely that 8.4 phosphate under all conditions is the eluate most favorable for the lead precipitate of field juice samples. In Table I, experiment 1, the appearance of an optimum concentration at M/1 for the elution of

TABLE III
ELUTION OF VIRUS FROM LEAD PRECIPITATE AT DIFFERENT pH VALUES

Exp. No.		Plants diseased of total inoculated				Relative susceptibility	Odds
		Readings from different flats			Total		
1	Juice	32/54	32/54	39/53	103/161	Juice>8.4	2:1
	pH 8.4	28/54	31/54	34/53	93/161	8.4>6.3	50,000:1
	pH 7.5	17/52	12/54	13/53	42/159	6.3>7.5	2:1
	pH 6.3	15/53	23/54	14/54	52/162		
2	Juice	19/54	16/54	20/54	55/162		
	pH 7.5	17/54	25/54	23/54	65/162	7.5>Juice	2:1
	pH 8.4	13/54	13/54	27/54	53/162	7.5>8.4	3:1
	pH 6.3	18/54	7/54	18/54	43/162	8.4>6.3	2.5:1
	pH 6.7	15/54	15/53	8/54	38/161	7.5>6.7	100:1

virus from the lead precipitate after treatment with acid phosphate at higher concentrations would indicate that the pH or concentration of the neutral phosphate buffer used may be determined by the pre-treatment with acid phosphate.

SUCCULENCE AND SUSCEPTIBILITY

In the relative concentration measurements by the method of Holmes (5) in which three flats of Turkish tobacco plants are inoculated with the same solution one flat may vary widely in the number of diseased plants. This variation has suggested the operation of some factor which, if controlled, would give greater accuracy to the concentration measurement or which might shed some light on the incidence or the progressive development of the disease. The degree of succulence is one respect in which the plants have been observed to vary.

Shade plants are generally more succulent, and in order to obtain a practical greenhouse shade for winter light intensities a glass cage was used. When the plants are grown within a glass cage in the greenhouse conditions have been attained under which the plants are more elongated and of more open cell structure. These plants are less susceptible than the ordinary greenhouse plants. The temperature within the glass cage was high but the wilting effect was counteracted by the high humidity resulting under these conditions of restricted ventilation.

In the first experiment the plants were inoculated on the fourth day after being placed in the cage. A maximum temperature of 120° F. was reached on only one day before the inoculation, with a maximum temperature of 90° to 105° the other days. Minimum day temperature was 80° and night temperature from 70° to 80° F.

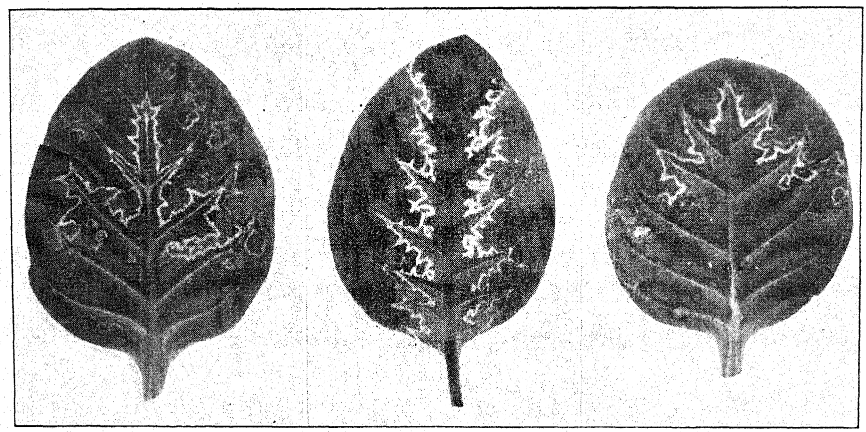

FIGURE 1. Abnormal symptoms of mosaic-diseased Turkish tobacco at high temperature.

In subsequent experiments later in the growing season the temperature was higher attaining a daily maximum of 120° F. Under these conditions of high temperature the plants are subject to severe wilting. To avoid loss the plants were taken from the cage on the fourth day after inoculation and removed to the greenhouse for symptoms to develop. The symptom shown in the diseased cage plants is a tendency for the leaf to be constricted near the tip, making the leaf triangular in shape as compared to the normal elongated heart shape of greenhouse leaves and the obovate shape of the undiseased high temperature leaf. Some of the diseased plants grown under high temperature conditions exhibit unusual symptoms. The pattern here resembles ring-spot, with irregular white patterns of plasmolyzed cells apparently tracing the outline of a serrate leaf (Fig. 1). Transfers from these plants, however, showed tobacco mosaic.

Johnson (6, 7) showed that the maximal temperature for activity of the virus was 30° to 36° C. At this temperature diseased plants lose their symptoms but the virus is still present as determined by the reappearance of symptoms when the plants are again held at lower temperatures. Newly inoculated plants held at this temperature did not develop symptoms. Johnson attributed this to a decreased rate of growth at the higher temperatures.

In Table IV, experiments 1, and part of 3, the odds indicate that the cage plants are less susceptible. In no case is this tendency reversed with significant odds.

TABLE IV
SUSCEPTIBILITY OF PLANTS GROWN IN CAGES AND IN GREENHOUSE

Experiment No.		Inoculated plants in	Plants diseased of total inoculated			Total	Relative susceptibility	Likelihood of a difference
1	a	Cage	9/51	20/50	25/51	54/152	G.H. > Cage	25:1
		Greenhouse	21/50	27/47	26/52	74/149		
	b	Cage	19/52	20/50	25/51	64/153	G.H. > Cage	20:1
		Greenhouse	21/54	32/53	35/54	88/161		
	c	Cage	4/35	7/47	9/53	20/135	G.H. > Cage	500:1
		Greenhouse	16/52	23/54	13/54	52/160		
2	a	Cage	31/46	30/44	10/22	71/112	Cage > G.H.	1:1
		Greenhouse	25/48	31/46		56/94		
	b	Cage	27/46	35/48	28/48	90/142	Cage > G.H.	8:1
		Greenhouse	20/52	28/52	35/54	83/158		
3	a	Cage	17/49	26/48	21/45	64/142	G.H. > Cage	5,000:1
		Greenhouse	36/49	29/50	39/50	104/149		
	b	Cage	38/51	37/47	33/48	108/146	Cage > G.H.	1:1
		Greenhouse	38/51	36/51	35/50	109/152		

Succulence is the factor presumed to be responsible for the difference demonstrated between cage and greenhouse plants. In order to determine whether cage plants had reached their maximum degree of succulence, at which stage it is desirable to inoculate, moisture determinations were made on cage plants. Both greenhouse and cage plants are high in water content. For the rapid determination of moisture, the weighed fresh leaves in aluminum boxes are plasmolyzed with ether (4), and water driven off on the hot plate under boiling toluene. By this method the bulk of the water is driven off in four hours and respiration is eliminated in the early stages of drying. The leaves are then dried in vacuo at 70° for 48 to 72-hour periods.

At the time of placing in the cage the average of 12 determinations on the plants that had grown in flats in the greenhouse gave 95.6 per cent water. Five days later the average of 12 determinations on the same plants after having been in the cage at a maximum daily temperature of 120° F. was again 95.6 per cent. The moisture content of greenhouse control plants was 94.7 per cent. (This compared with 87 to 90 per cent in field leaves.) For following the changes in degree of succulence as a preliminary to picking the stage of greater succulence of the cage plants this method is too long drawn out. Even with a shorter method for determining moisture the plants would have passed the stage before it could be recognized. The contrast between cage and greenhouse plants may best be obtained in microscopic section. The cell wall of the leaves of cage plants has been partially liquified at the higher temperature so that the leaves section with difficulty and cells appear widely dispersed.

LEAF EXPANSION AND SUSCEPTIBILITY

In addition to succulence as the possible underlying factor in the difference in susceptibility the rate of growth or rate of leaf expansion was considered. By measuring the expansion of the scar in the leaf caused by the five pin punctures it was possible to get an index of the rate of expansion of the leaf in the vicinity of inoculation. When the inoculated leaves are grouped in classes according to the expansion of the inoculation scar, then if the rate of growth is a positive factor in susceptibility a greater number of diseased plants should occur among the groups of greater expansion.

For cage and greenhouse plants the measurements were obtained on the fourth and fifth days after inoculation. For inoculation the procedure regularly followed was to select a young leaf, usually the second or third from the bud. The inoculation was made between the third and fourth lateral veins, in a region that expands rapidly. By using dividers the widths of the scars were obtained without disturbing the plants. The measurements were recorded with the position of the plant in the flat; cage plants were returned to the cage until ready to be counted.

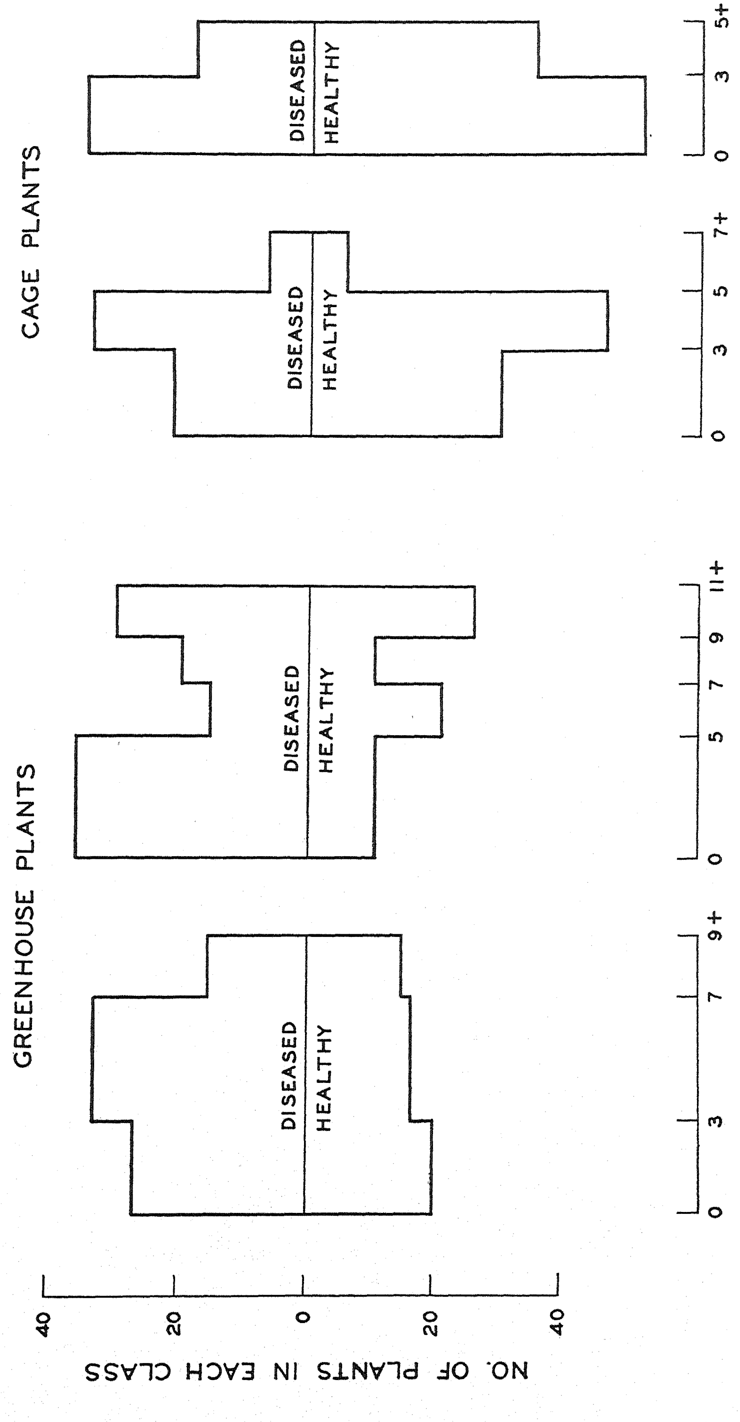

FIGURE 2. Uniform distribution in classes according to rate of growth (leaf scar expansion) of diseased and healthy plants in series of 150 inoculated plants grown in the greenhouse and in high temperature cage.

The histograms (Fig. 2) represent the distributions of healthy and diseased plants in classes according to leaf expansion. There is no tendency for the diseased plants to be grouped among the classes of greater or lesser expansion. The distribution is entirely random. In adjacent plants the rate of growth is not a factor in susceptibility.

SUMMARY

The following improvements in the Vinson and Petre lead precipitation method for the separation and purification of mosaic virus from tobacco leaves were made:

(1) Increasing the concentration of potassium acid phosphate in the preliminary elution assisted in removing the pigment.

(2) Increasing the acidity of the potassium acid phosphate buffer in the preliminary elution increased the activity of the virus removed in the final eluate but was less effective in removing pigment.

(3) In the final elution, the lead acetate precipitates from juice of field-grown tobacco plants required an alkaline phosphate buffer of higher pH than 6.5 to demonstrate their full activity. Favorable elution was obtained at pH 7.5 and 8.4.

Tobacco plants grown within glass cages were found to be less susceptible to infection with tobacco mosaic than plants grown in the greenhouse. The cage-grown plants were also more succulent and this succulence is presumed to be responsible for the difference between cage-grown and greenhouse-grown plants.

Rate of leaf expansion was found not to be a factor in susceptibility.

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FURTHER EXPERIMENTS ON ORGANIC THIOCYANATES AS INSECTICIDES[1]

FRANK WILCOXON AND ALBERT HARTZELL

In a previous publication (3) it was shown that certain organic thiocyanates exhibit marked toxicity to *Aphis rumicis* L., the citrus mealy bug (*Pseudococcus citri* Risso), and the red spider mite (*Tetranychus telarius* L.). The present paper is a continuation of this investigation, in which a study has been made of the tolerance of 75 species and varieties of plants to two organic thiocyanates which showed promise as insecticides. These two thiocyanates were tested on 12 species of insects and mites. In addition to this a comparison was made of the toxicity to *Aphis rumicis* of five closely related thiocyanates, of which two were isomeric, in order to find out how slight changes in structure influenced toxicity.

PREPARATION OF COMPOUNDS USED

Trimethylene dithiocyanate. This was prepared from trimethylene bromide (Eastman) and KSCN as described for γ-thiocyanopropyl phenyl ether in a previous publication (3). *Analysis.* Calc. for $C_5H_6N_2S_2$: C, 37.93. H, 3.86. Found: C, 38.3. H, 3.80. M. Pt. 18° C. Uncorr.

Ethylene dithiocyanate. Prepared as above from ethylene dibromide. *Analysis.* Calc. for $C_4H_4N_2S_2$: C, 33.22. H, 2.98. Found: C, 33.1. H, 2.94. M. Pt. 90° C. Uncorr.

Propylene dithiocyanate. (Isomeric with trimethylene dithiocyanate) Prepared as above from propylene dibromide. *Analysis.* Calc. for $C_5H_6N_2S_2$: S, 40.79. Found: 40.8.

Ethylene chlorothiocyanate. Prepared from ethylene chlorobromide. *Analysis.* Calc. for C_3H_4NSCl: S, 26.37. Found: S, 26.48.

γ-thiocyanopropyl phenyl ether. The preparation of this substance has been described previously (3).

Trimethylene chlorothiocyanate. From trimethylene chlorobromide and KSCN. *Analysis.* Calc. for C_4H_6NSCl: S, 23.57. Found: S, 23.67.

COMPARATIVE TOXICITY TO APHIS RUMICIS

The method of testing was the same as described previously (2). Each of the above mentioned compounds was used at a concentration of 0.1 per cent, with 0.5 per cent Penetrol. The results of the test are shown in Table I. It is evident that trimethylene dithiocyanate is the most toxic, and the only one to give satisfactory control at this concentration. This compound was, therefore, compared with the one which had been found most satisfactory previously, namely γ-thiocyanopropyl phenyl ether.

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 86.

TABLE I

COMPARATIVE TOXICITY TO APHIS RUMICIS OF ORGANIC THIOCYANOGEN COMPOUNDS

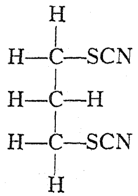
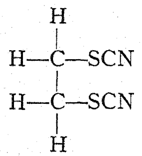
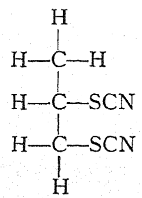
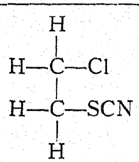
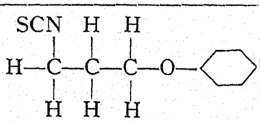
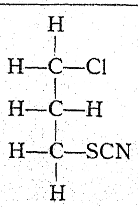
Formula	Name	Compound + spreader, per cent dead
	Trimethylene dithiocyanate	99.3 98.8
	Ethylene dithiocyanate	48.5 65.4
	Propylene dithiocyanate	80.4 78.0
	Ethylene chlorothiocyanate	58.5 78.5
	γ-thiocyanopropyl phenyl ether	98.0 93.7
	Trimethylene chlorothiocyanate	55.4 56.0

TABLE II

COMPARATIVE TOXICITY OF ORGANIC THIOCYANATES TO INSECTS AND MITES

Name of insect or mite	Developmental stage	Conc. of thiocyanate used, %	γ-thiocyanopropyl phenyl ether + 0.5% Penetrol		Trimethylene dithiocyanate + 0.5% Penetrol	
			No. individuals tested	% dead av.	No. individuals tested	% dead, av.
Red spider mite (*Tetranychus telarius* L.)	Larva and adult	0.10	235	98.0	100	100.0
Bean aphid (*Aphis rumicis* L.)	Agamic	0.02	204	73.0	146	80.0
Bean aphid (*Aphis rumicis* L.)	Agamic	0.08	181	91.2	265	93.2
Melon aphid (*Aphis gossypii* Glover)	Agamic	0.10	250	100.0	250	100.0
Potato aphid (*Illinoia solanifolii* Ashmead)	Agamic	0.10	861	81.7	115	86.1
Citrus mealy bug (*Pseudococcus citri* Risso)	Larva and adult	0.10	250	100.0	104	100.0
Long-tailed mealy bug (*Pseudococcus adonidum* L.)	Larva and adult	0.10	250	100.0	250	100.0
Black blister beetle (*Epicauta pennsylvanica* De Geer)	Adult	0.10	110	6.4	100	12.0
Lesser European bark beetle (*Scolytus multistriatus* Marsh.)	Adult	0.10	250	100.0	100	100.0
Mexican bean beetle (*Epilachna corrupta* Muls.)	Larva	0.10	72	38.8	100	27.0
Mexican bean beetle (*Epilachna corrupta* Muls.)	Larva	0.20	100	76.0	100	32.0
Mexican bean beetle (*Epilachna corrupta* Muls.)	Adult	0.10	67	35.8	50	50.6
Mexican bean beetle (*Epilachna corrupta* Muls.)	Adult	0.20	100	37.0	109	79.8
Potato flea beetle (*Epitrix cucumeris* Harris)	Adult	0.20	200	98.0	100	100.0
Rose chafer (*Macrodactylus subspinosus* Fabr.)	Adult	0.10	100	0	—	—
Rose chafer (*Macrodactylus subspinosus* Fabr.)	Adult	0.20	50	0	—	—
Fall canker worm (*Alsophila pometaria* Harris)	Larva	0.05	37	10.8	—	—
Fall canker worm (*Alsophila pometaria* Harris)	Larva	0.20	35	42.8	—	—

COMPARATIVE TOXICITY OF TRIMETHYLENE DITHIOCYANATE AND γ-THIOCYANOPROPYL PHENYL ETHER

Comparative tests were made using the above two compounds on 11 species of insects (representing three orders) and one species of mite. The compounds were applied as contact sprays in the laboratory. In most cases several concentrations of the toxic constituent were used. The results appear in Table II.

The method of testing the melon aphid (*Aphis gossypii* Glover) and the potato aphid (*Illinoia solanifolii* Ashmead) was the same as previously described (2) for *Aphis rumicis*, except for the host plant used. *Aphis gossypii* was tested on Easter lily (*Lilium longiflorum* Thunb. var. *eximium* Nichols.) and *Illinoia solanifolii* on potato (*Solanum tuberosum* L.) and dahlia (*Dahlia variabilis* Desf.). Peach seedlings (*Prunus persica* [L.] Stokes) infested with red spider mite were sprayed in a similar manner, the leaves were detached after spraying and placed in moist chambers, and counts were made of the living and dead mites at intervals of 24 and 36 hours. Cotton plants (*Gossypium hirsutum* L.) and shining club moss (*Lycopodium lucidulum* Michx.) infested with the citrus mealy bug and the long-tailed mealy bug (*Pseudococcus adonidum* L.) were sprayed similarly, but the leaves in most cases were not detached from the plants until the counts were made.

The black blister beetle (*Epicauta pennsylvanica* De Geer), the lesser European bark beetle (*Scolytus multistriatus* Marsh.), the Mexican bean beetle (*Epilachna corrupta* Muls.), the rose chafer (*Macrodactylus subspinosus* Fabr.), and the fall canker worm (*Alsophila pometaria* Harris) were placed in beakers and sprayed by means of a hand atomizer (De Vilbiss No. 15). They were removed immediately to cages containing their unsprayed host plants. Flea bettles (*Epitrix cucumeris* Harris) were placed in inverted battery jars and sprayed by introducing the nozzle of the atomizer from beneath. Unsprayed potato leaves were supplied for food. Counts of living and dead bettles were made at intervals of 24 and 36 hours.

Inspection of Table II indicates that both compounds are specific in their action. Marked toxicity was observed for the red spider mite, *Aphis rumicis*, the melon aphid, the citrus mealy bug, the long-tailed mealy bug, the lesser European bark beetle, and the potato flea beetle with both compounds at concentrations of 0.1 or 0.08 per cent. With the possible exception of the potato aphid, the sprays proved unsatisfactory for the other species of insects tested. Beetles of small size were more readily killed by the sprays than beetles of large size.

The possible action of organic thiocyanates as stomach poisons has not been investigated in the present study.

PLANT TOLERANCE

Seventy-five species and varieties of plants were tested with regard to their tolerance to trimethylene dithiocyanate and γ-thiocyanopropyl phenyl ether. The results appear in Table III.

Plants growing in the field were sprayed by means of a hand sprayer while greenhouse plants were sprayed as described previously for nasturtium in toxicity tests. These experiments were conducted during August 1934, when the temperature was relatively high. Applications were made during the morning hours in order that the plants would become dry before the maximum temperature of the day was reached. The plants were

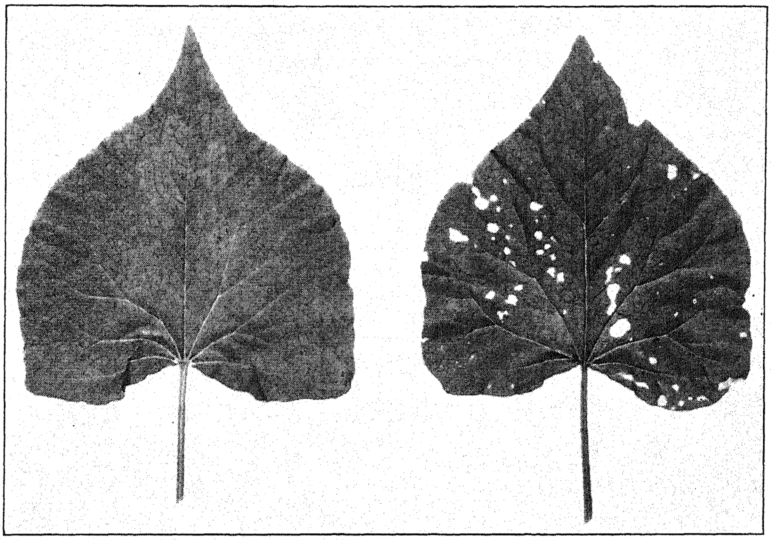


FIGURE 1. Effect of a solution of γ-thiocyanopropyl phenyl ether (0.1 per cent) and 0.5 per cent Penetrol sprayed on buckwheat leaf. Note bleached areas. On left, leaf sprayed with 0.5 per cent Penetrol, uninjured.

examined for injury 24 hours after spraying and at the end of seven days. If no injury was observed at the end of that time the plant was considered tolerant. If rain fell during the period, the experiment was repeated. Potted plants in the greenhouse were watered twice daily without wetting the leaves.

The temperature out-of-doors ranged from 52° to 89° F., with a mean temperature of 72.0° F., and a mean relative humidity of 68.4 per cent for the month (1). The rainfall was light with 2.77 inches of precipitation during the month. The temperature of the greenhouse ranged from 68° to 100° F., with approximately 50 to 70 per cent relative humidity.

TABLE III

SENSITIVITY OF HOST PLANTS TO ORGANIC THIOCYANATE SPRAYS

Name of plant	No. plants tested	Plant height, inches
Plants intolerant to both γ-thiocyanopropyl phenyl ether and trimethylene dithiocyanate		
Columbine (*Aquilegia* sp.)	2	6
Pepper (*Capsicum frutescens* L.)**†	2	10
Phlox, perennial (*Phlox* sp.)*	2	36
Poppy, California (*Eschscholzia californica* Cham.)*	2	18
Soy bean (*Glycine max* Merr.) (Old plants)**†	8	18
Sunflower (*Helianthus debilis* Nutt.)**†	2	8
Plants intolerant to trimethylene dithiocyanate and tolerant to γ-thiocyanopropyl phenyl ether		
Cabbage (*Brassica oleracea* L. var. *capitata* L.)**	2	7
Calendula (*Calendula officinalis* L.)*	2	20
Cockscomb (*Celosia argentea* L. var. *cristata* Kuntze)*	2	22
Rose (*Rosa* sp. var. Ariel)	2	14
Verbena, perennial (*Verbena* sp.)*	2	14
Plants intolerant to γ-thiocyanopropyl phenyl ether and tolerant to trimethylene dithiocyanate		
Beet (*Beta vulgaris* L.)**†	8	6
Buckwheat (*Fagopyrum esculentum* Moench.) (Young and old plants)**	10	24
Canna (*Canna generalis* Bailey)	2	24
Coleus (*Coleus blumei* Benth.) (Young plants)	2	10
Easter lily (*Lilium longiflorum* Thunb. var. *eximium* Nichols.)**	2	21
Holly (*Ilex opaca* Ait.)	2	18
Larkspur (*Delphinium ajacis* L.)*	2	24
Rose (*Rosa* sp. var. Paul's Scarlet)	2	84
Snapdragon (*Antirrhinum majus* L.)*	2	14
Swiss chard (*Beta vulgaris* L. var. *cicla* L.)**†	8	6
Plants tolerant to both γ-thiocyanopropyl phenyl ether and trimethylene dithiocyanate		
Achillea (*Achillea ageratum* L.)*	2	20
Balsam (*Impatiens balsamina* L.) (Young plants)**†	2	24
Bean (*Phaseolus vulgaris* var. *humilis* Alef.)**	8	18
Carnation (*Dianthus caryophyllus* L.)	2	8
Chrysanthemum (*Chrysanthemum morifolium* Ram.)	2	14
Coleus (*Coleus blumei* Benth.) (Young plants)**	2	8
Coreopsis (*Coreopsis* sp.)*	2	24
Corn (*Zea mays* L.)	2	18
Cornflower (*Centaurea cyanus* L.)*	2	28
Cotton (*Gossypium hirsutum* L.) (Young plants)**	6	28
Cranberry (*Vaccinium macrocarpon* Ait.)**	2	32
Cucumber (*Cucumis sativus* L.)**	4	10
Currant (*Ribes sativum* Syme)	1	30
Dahlia (*Dahlia variabilis* Desf. var. Jersey's Beauty)	2	32
Dahlia (*Dahlia variabilis* Desf. var. Mrs. I. de Ver Warner)	2	46
Digitalis (*Digitalis purpurea* L.)	2	6

TABLE III (*Continued*)

Name of plant	No. plants tested	Plant height, inches
Plants tolerant to both γ-thiocyanopropyl phenyl ether and trimethylene dithiocyanate (*continued*)		
Eggplant (*Solanum melongena* L. var. *esculentum* Nees.)	2	20
English ivy (*Hedera helix* L.)	2	36
Gardenia (*Gardenia jasminoides* Ellis)**	1	8
Geranium (*Pelargonium hortorum* Bailey)	2	14
Gerbera (*Gerbera jamesoni* Bolus)*	2	26
Gladiolus (*Gladiolus* sp.)	2	14
Gooseberry (*Ribes grossularia* L.)	1	20
Helianthus (*Helianthus multiflorus* Hort.)*	2	56
Hydrangea (*Hydrangea paniculata* Sieb. var. *grandiflora* Sieb.)*	2	18
Iris (*Iris* sp.)	2	20
Kale (*Brassica oleracea* L. var. *acephala* DC.)**	4	6
Lobelia (*Lobelia* sp.)*	2	8
Maple, Japanese (*Acer palmatum* Thunb.)**	1	26
Marigold, African (*Tagetes erecta* L.)*	2	24
Mexican fire-plant (*Euphorbia heterophylla* L.)	2	8
Nasturtium (*Tropaeolum minus* L.)**	20	6
Pansy (*Viola tricolor* L. var. *hortensis* DC.)*	2	8
Peach seedlings (*Prunus persica* [L.] Stokes)**†	4	12
Peas, field (*Pisum sativum* L. var. *arvense* Poir.)**†	8	24
Pentstemon (*Pentstemon* sp.)*	2	34
Peony (*Paeonia* sp.)	1	36
Petunia (*Petunia axillaris* BSP.)*	2	14
Phlox, annual (*Phlox drummondii* Hook.)*	2	16
Potato (*Solanum tuberosum* L.)	4	18
Rose (*Rosa* sp. var. Excelsa)	2	96
Rose (*Rosa* sp. var. Francis Gaunt)	2	14
Rose (*Rosa* sp. var. Red Radiance)*	2	29
Rose-of-Sharon (*Hibiscus syriacus* L.)*	1	48
Rudbeckia (*Rudbeckia triloba* L.)*	2	50
Salvia (*Salvia splendens* Ker.)	2	14
Scabiosa (*Scabiosa atropurpurea* L.)*	2	20
Shining club moss (*Lycopodium lucidulum* Michx.)**	10	5
Stock (*Mathiola incana* R. Br.)	2	8
Stokesia (*Stokesia laevis* Greene)	2	14
Tomato (*Lycopersicon esculentum* Mill. var. Bonny Best)	2	30
Turnip (*Brassica rapa* L.)**	8	6
Verbena, annual (*Verbena* sp.)	2	12
Zinnia (*Zinnia elegans* Jacq.)*	2	14

* Plant in bloom.
** Greenhouse tests.
† Injured by Penetrol alone.

Inspection of Table III shows that trimethylene dithiocyanate was toxic to 11 species and varieties of plants while 16 species and varieties were sensitive to γ-thiocyanopropyl phenyl ether. The injury in the latter case was more severe in nearly every instance. Since in a number of cases the degree of injury depends on the age of the plants, heights are indicated in Table III. Young cotton, balsam, and coleus plants were not injured by either compound when used at a concentration of 0.1 per cent, while

old plants were injured. In a number of cases the plants were sensitive to the spreader when used alone, so it is possible that some of the injury might have been avoided by the selection of a less toxic emulsifying agent.

Spray applications of γ-thiocyanopropyl phenyl ether (0.1 per cent) and 0.5 per cent Penetrol produced bleached necrotic spots on the leaves of buckwheat plants (Fig. 1). Check plants sprayed with 0.5 per cent Penetrol were uninjured. Trimethylene dithiocyanate (0.1 per cent) and Penetrol as well as γ-thiocyanopropyl phenyl ether produced brown necrotic areas on the leaves of old soybean plants.

The number of tests that could be made were restricted due to the difficulty of producing large quantities of the toxic compounds in the laboratory. The results on plant tolerance are presented, therefore, as preliminary.

SUMMARY

Five closely related organic thiocyanates have been prepared and their insecticidal properties compared with that of γ-thiocyanopropyl phenyl ether previously tested. Of these compounds one, namely trimethylene dithiocyanate, was equal to or better than the above mentioned ether, while the remainder were distinctly inferior. Trimethylene dithiocyanate was considerably more toxic than its isomer, propylene dithiocyanate.

Trimethylene dithiocyanate controlled *Aphis rumicis*, the melon aphid (*Aphis gossypii*), the citrus mealy bug (*Pseudococcus citri*), the long-tailed mealy bug (*Pseudococcus adonidum*), the lesser European bark beetle (*Scolytus multistriatus*), the potato flea beetle (*Epitrix cucumeris*), and the red spider mite (*Tetranychus telarius*).

Of 75 species and varieties of plants tested with regard to their tolerance to trimethylene dithiocyanate (0.1 per cent) 64 were tolerant as compared with 59 for γ-thiocyanopropyl phenyl ether used at the same concentration.

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RELATION OF VIRUS CONCENTRATION TO THE NUMBER OF LESIONS PRODUCED

W. J. Youden, Helen Purdy Beale, and John D. Guthrie

INTRODUCTION

It is known that several filterable viruses of plants produce local necrotic lesions when rubbed on the leaves of certain hosts. In general, decreasing concentrations of these virus extracts induce relatively fewer lesions. Various factors have been regarded as important in influencing the number of lesions resulting from an inoculation. The relation of the concentration of virus to the number of lesions produced has been studied by several workers. So little is known about the process that apparently no mechanism has been postulated in sufficient detail to warrant the formulation of any quantitative relationships that might be subjected to experimental inquiry. The difficulty, depending upon the experimental results to suggest the relationship, lies in the fact that the results may not be examined from the proper point of view. Thus it may be a *convenience* to plot one of the variables on a logarithmic scale. It by no means follows that such a scale will bring out the essential nature of the relationship between the factors.

In the event that theoretical guidance as to the character of the relationship is lacking, the results are often plotted in the experimental units. It is usually not possible to deduce the type of function by an inspection of a curve drawn free-hand through several points. Especially is this true if the experimental error is large. The outstanding exception is a linear relationship. Consequently frequent efforts are made to plot the results in such a way that a straight line is obtained. A favorite device is that of plotting the logarithms of one or both of the variables. There are, however, many choices available. One variable may be plotted against the reciprocal, or the square of the other.

Given some basis for believing the function is a particular type it is possible to test the feasibility of the hypothesis. This may lead to a definite rejection of the idea or to the conclusion that the experimental data available are not inconsistent with the assumed relationship. Rarely is a simple exact relationship found. A simple function naturally is one in which a very limited number of variables or factors are taken into consideration. The fact that some are left out need not imply that they do not operate but only that their rôle is minor, or perhaps less than the experimental error.

The purpose of this paper is to show that data at present available are not inconsistent with the concept that the number of lesions may be ex-

pressed as a simple function of the dilution and two constants, to each of which physical counterparts may be assigned. The data available consist of dilution curves taken from six different sources using four viruses on three different test plants.

EXAMINATION OF PUBLISHED DILUTION CURVES

Figure 1 shows typical dilution curves published by Holmes (6), Samuel and Bald (10), and Beale (1). Holmes plotted the lesion counts against the logarithm of the dilution. Price (8, 9) followed the same practice and his curves are similar to curve A which is taken from Holmes' paper (6). Samuel and Bald (10) preferred to plot the logarithms of the variables; one of their figures is reproduced as curve B. Beale (1) used a linear scale for both the counts and the dilution obtaining the curve C. The corresponding curves AA, BB, and CC show the appearance of the curves obtained when the function

$$y = N(1 - e^{-ax})$$

y = number of lesions

x = concentration = reciprocal of dilution

is plotted using the different schemes adopted by the several workers. In every case the parallelism between the experimental curve and the theoretical one is striking. The upper tip of the curve AA represents a region of concentrations not covered by the Holmes curve. There is also a difference between the slopes of the straight line portions of curves B and BB. Data obtained from Beale (1), Chester (3), Caldwell (2), and Price (8, 9) confirm the theoretical slope of unity instead of approximately 0.6 given by Samuel and Bald (10). In the same paper Samuel and Bald (10, p. 79) report a slope of about 5/6 for the dilution curves of tomato spotted wilt. The curves shown in Figure 1 furnish qualitative support for the function given above. Once the function is known it is clear that none of these three methods of representing the data can possibly result in a straight line over the whole range of dilutions.

ANALOGY WITH BACTERIA COUNTS

Consider a very dilute suspension of bacteria—say one that averages one per cubic centimeter. If one cubic centimeter of the suspension is added to each of N Petri dishes containing sterile agar and then incubated a certain number will be found sterile, about an equal number with one colony, and an approximately equal number to have two or more colonies. Of course a more dilute suspension would give a larger fraction of sterile plates. This situation is encountered in practice in the process of isolating pure cultures. The proportion of sterile plates is governed by the average

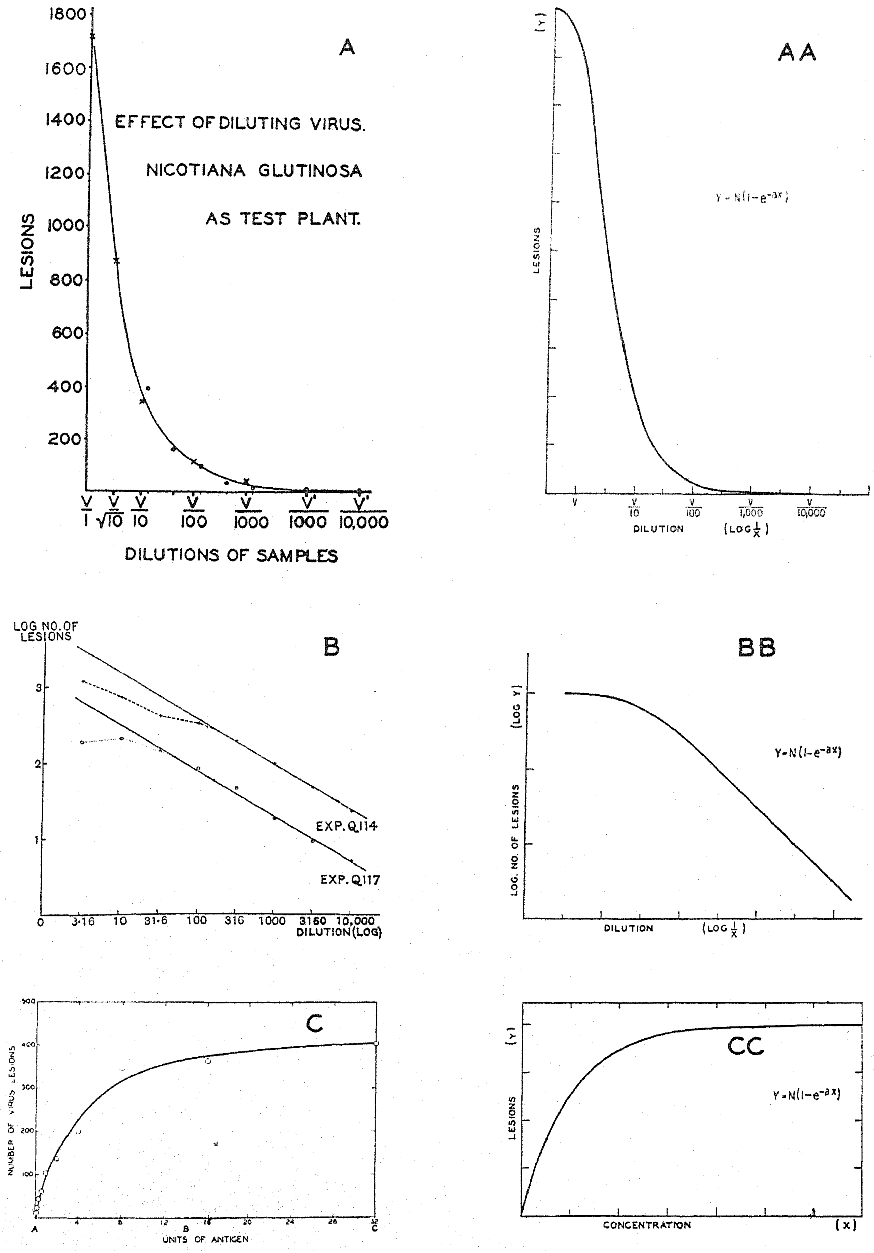

FIGURE 1. Comparison of curves taken from the literature with graphs of the function $y = N(1 - e^{-ax})$. The form of the experimental curves depends upon the method adopted for plotting the results. The equation gives the same curves when plotted in the same way.

concentration of bacteria in the suspension. It is possible to calculate the proportion in advance by evaluating the quantity e^{-a} where $e = 2.718$ (the base for natural logarithms), and a is the average number of bacteria in the volume added to each dish (4, 5). Ordinarily the concentration is not known. It may be determined by noting the fraction sterile and finding from tables of the exponential that value of a which gives e^{-a} the value observed. The functional relationship is

$$p = e^{-ax}$$

where p is the fraction or proportion of *sterile* plates and x any fractional concentration of the original suspension. If N plates are used and z is the number *sterile* then

$$\frac{z}{N} = e^{-ax} \quad \text{or} \quad z = Ne^{-ax}.$$

Suppose an experiment is done with two different dilutions of a suspension containing a bacteria per cc. using the same number of plates N in each case. Further suppose that the number N is not disclosed. Two different numbers z_1 and z_2 of sterile plates will be found corresponding to the two different dilutions x_1 and x_2. Two equations

$$z_1 = Ne^{-ax_1}$$

and

$$z_2 = Ne^{-ax_2}$$

are available in which N and a are unknown. These may be evaluated by solving the equations. In other words, if the test is done with two or more dilutions it is not necessary to know the total number, N, of plates.

Should it be desired to test this relationship experimentally it is evident that the logarithm of the number of *sterile* plates is a linear or straight line function of the dilution, x,

$$\log z = \log N - ax \log e$$

or

$$\log z = N' - a'x.$$

Also if data for a number of dilutions are available the constants N' and a' may be evaluated by the method of least squares and then converted to N and a.

The equations have been set up in terms of z, the number of *sterile* plates. Let y equal the number of plates showing colonies. Then $z = N - y$, and the equations may be expressed in terms of y by making this substitution

$$N - y = Ne^{-ax} \quad \text{or} \quad \frac{N - y}{N} = e^{-ax}.$$

If the total number N is not disclosed and instead of z, the number sterile, y, the number showing colonies, is furnished, the evaluation of the

constants is not nearly so simple. It is not possible to obtain the logarithm of $N-y$ since N is unknown and one of the constants that is sought. If the equation is solved for y there results

$$y = N(1 - e^{-ax}).$$

Here the logarithm of y is not a linear function of x. This is a somewhat awkward function to handle. One device is to substitute for e^{-ax} the power series

$$1 - ax + \frac{a^2x^2}{2!} - \frac{a^3x^3}{3!} + .$$

Then

$$y = N\left(ax - \frac{a^2x^2}{2!} + \frac{a^3x^3}{3!} - \cdots\right).$$

For small values of x the higher powers are negligible and

$$y = Nax \quad \text{or} \quad y/N = ax.$$

From this it would be predicted that the number of plates showing colonies would for very *dilute* suspensions be proportional to the concentration of bacteria. Taking the logarithm of both sides gives the equation

$$\log y = \log (Na) + \log x.$$

In this dilute range the logarithms of x and y are therefore in a linear relation to each other. Since the coefficient of $\log x$ is unity the line will have unit slope.

The virus situation is comparable to the above if the susceptible areas are considered analogous to the plates and virus particles to the bacteria. Stated in another way, the equation

$$y = N(1 - e^{-ax})$$

is what one would expect the data to follow if the lesions resulted from a random distribution of virus particles on a number of susceptible areas, each area receiving one or more particles forming a lesion. Here y is the number of lesions obtained with any given concentration x of virus. N represents the maximum number of lesions obtainable—possibly the number of susceptible areas available on the leaves. The constant a is a property of the virus preparation—literally it is, for the undiluted extract, the average number of infective particles per susceptible area.

This explains why no simple relationship was found so long as the number of lesions was chosen as the variable to plot against dilution. It is the number of lesions that do *not* come out that follow a simple exponential law. The observations necessarily are made on the lesions which do appear. This merely adds to the arithmetical labor since the method of evaluating the constants N and a is more tedious given the y values instead of the z values.

METHOD OF DETERMINING CONSTANTS OF THE EQUATION

Each dilution and the corresponding count furnish a pair of values for x and y which upon substitution in the equation leaves an equation in N and a. The number of such equations available is determined by the number of dilutions that have been made. The problem is to solve these several equations for N and a. The best values for N and a are those which reduce to a minimum the sum of the squares of the differences between the actual counts and the counts calculated from the equation using the determined values of N and a. Since the usual formulas cannot be used, an example is given in detail. It is necessary to make estimates of N and a and then obtain corrections for these estimates. If the first estimates have been fortunate the corrections will be small, otherwise it may be desirable to repeat the process using the improved estimates.

The column headings in Table I indicate the operations performed upon the original data listed under x and y. Halvorson and Ziegler's Quantitative Bacteriology (5) or the Smithsonian Tables (12) contain tables of the exponential required in column 4. The seventh column under the heading r lists the differences between experimental y and calculated y found in column 6. Care must be observed with respect to the signs. The values tabulated in columns 5, 7, and 8 form the coefficients in a set of residual equations. The variables in these equations are ΔN and Δa, the corrections

TABLE I

METHOD OF CALCULATING a AND N; a ESTIMATED EQUAL TO 3.125, N ESTIMATED EQUAL TO 65

x	y	ax	e^{-ax}	$1-e^{-ax}$	$N(1-e^{-ax})$	r	Nxe^{-ax}
1.000	62.1	3.125	.0439	.9561	62.15	.05	2.854
.200	30.8	.625	.5353	.4647	30.21	−.59	6.959
.040	7.0	.125	.8825	.1175	7.64	.64	2.295
.008	.5	.025	.9753	.0247	1.61	1.11	.507

$$\left.\begin{aligned}
V_1 &= 2.854\ \Delta a + .9561\ \Delta N + .05\\
V_2 &= 6.959\ \Delta a + .4647\ \Delta N - .59\\
V_3 &= 2.295\ \Delta a + .1175\ \Delta N + .64\\
V_4 &= .507\ \Delta a + .0247\ \Delta N + 1.11
\end{aligned}\right\}\text{Residual equations}$$

$$\begin{aligned}
8.14532\ \Delta a &+ 2.72871\ \Delta N + .1427\\
48.42768\ \Delta a &+ 3.23385\ \Delta N - 4.1058\\
5.26703\ \Delta a &+ .26966\ \Delta N + 1.4688\\
.25705\ \Delta a &+ .01252\ \Delta N + .5628
\end{aligned}$$

$$\left.\begin{aligned}
62.0971\ \Delta a + 6.24474\ \Delta N - 1.9315 &= 0\\
6.24474\ \Delta a + 1.14449\ \Delta N - .12375 &= 0
\end{aligned}\right\}\text{Normal equations}$$

$$\Delta a = .049 \qquad \Delta N = -.14$$
$$a + \Delta a = 3.174 \qquad N + \Delta N = 64.86$$

sought to the estimates N and a. From these equations a new set is formed by multiplying each equation by the coefficient of Δa in that equation.

The new set is summed giving one equation. A second equation results from repeating the process using the coefficient of ΔN as a multiplier. The two normal equations may then be solved in any desired manner for ΔN and Δa. In practice the work of multiplying and adding is carried through on a machine and the intermediate equations left unwritten. This calculation is described by Scarborough (11).

ILLUSTRATIONS SHOWING HOW CLOSELY THE THEORETICAL EQUATION FITS THE DATA

A more exacting test of this functional relationship can now be applied. If the function describes the phenomenon, then, for the data available, it should be possible to obtain values for the constants N and a. The function may be written

$$\frac{N - y}{N} = e^{-ax}$$

or

$$\log \frac{N - y}{N} = - ax \log e.$$

After N is known the expression on the left of the last equation may be easily evaluated and plotted against corresponding values of x. The points, subject to experimental variation, should lie along a straight line. This has been done for a set of data from each of four sources and the resulting straight lines are shown in Figure 2. Figure 3 shows the same data plotted with linear scales and the smooth curves plotted from the equation

$$y = N(1 - e^{-ax})$$

using the appropriate values for N and a in each case. These curves are identical and are made to coincide by allowing the same amount of ordinate for each N and by using the same scale expressed in ax units for the x-axis. The curves have been plotted separately in order to establish clearly that the data for any dilution curve establish the two constants required. The constants are given in the equation associated with each curve. Figure 4 shows the data from several other dilution curves plotted on the same axes. The lesion counts are expressed as a fraction of N for each curve so that the y-scale runs from zero to one. The data used are listed in Table II. This table also gives the estimates of the constants and the lesion counts for each dilution calculated from these constants. This makes possible a direct comparison between the experimental counts (column 3) and the calculated values (column 4). The particular virus and host plant are also recorded and wherever possible the number of plants or leaves used in the experimental work. The values given for N and a are those obtained when the correction did not exceed five per cent of the preliminary esti-

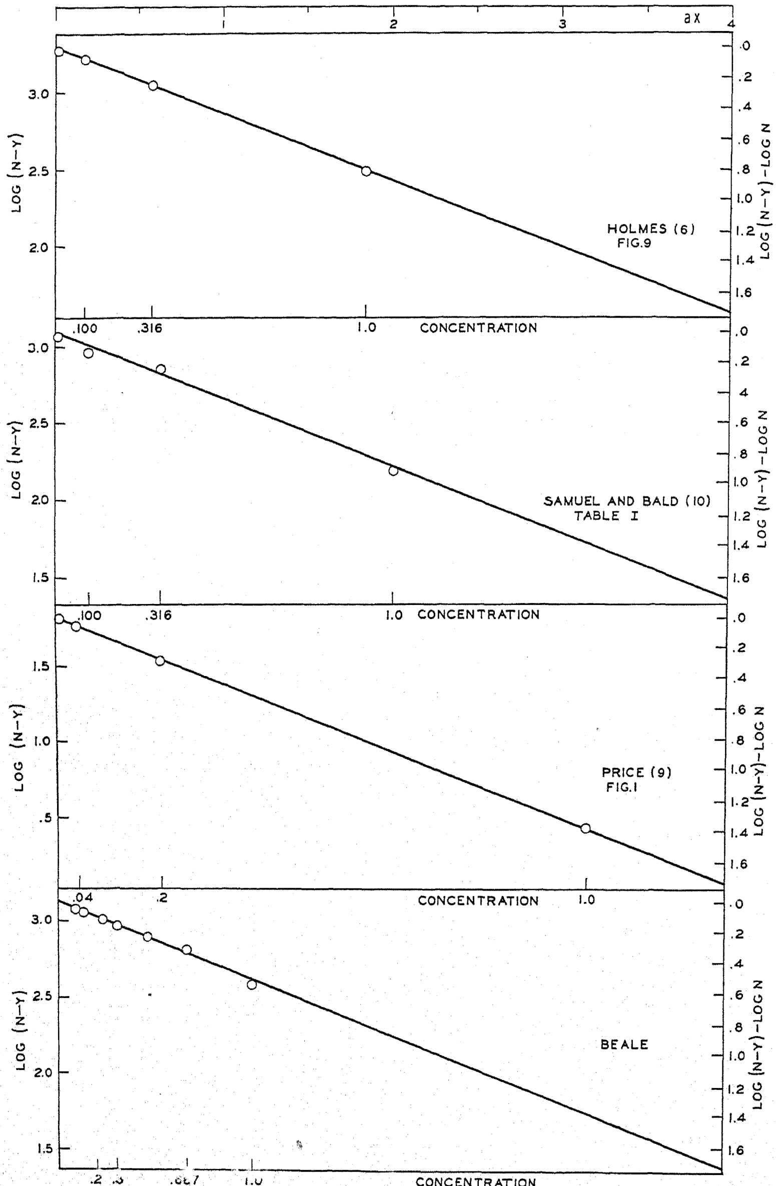

FIGURE 2. The four curves show how well experimental points lie on a straight line when the method of plotting is that required to give a straight line for the theoretical equation. The scale of ax units at the top applies to all the curves. The right hand scale of ordinates is also the same for each curve. The point marked 1.0 on each abscissa is plotted on the ax scale at the value obtained for a for each set of data.

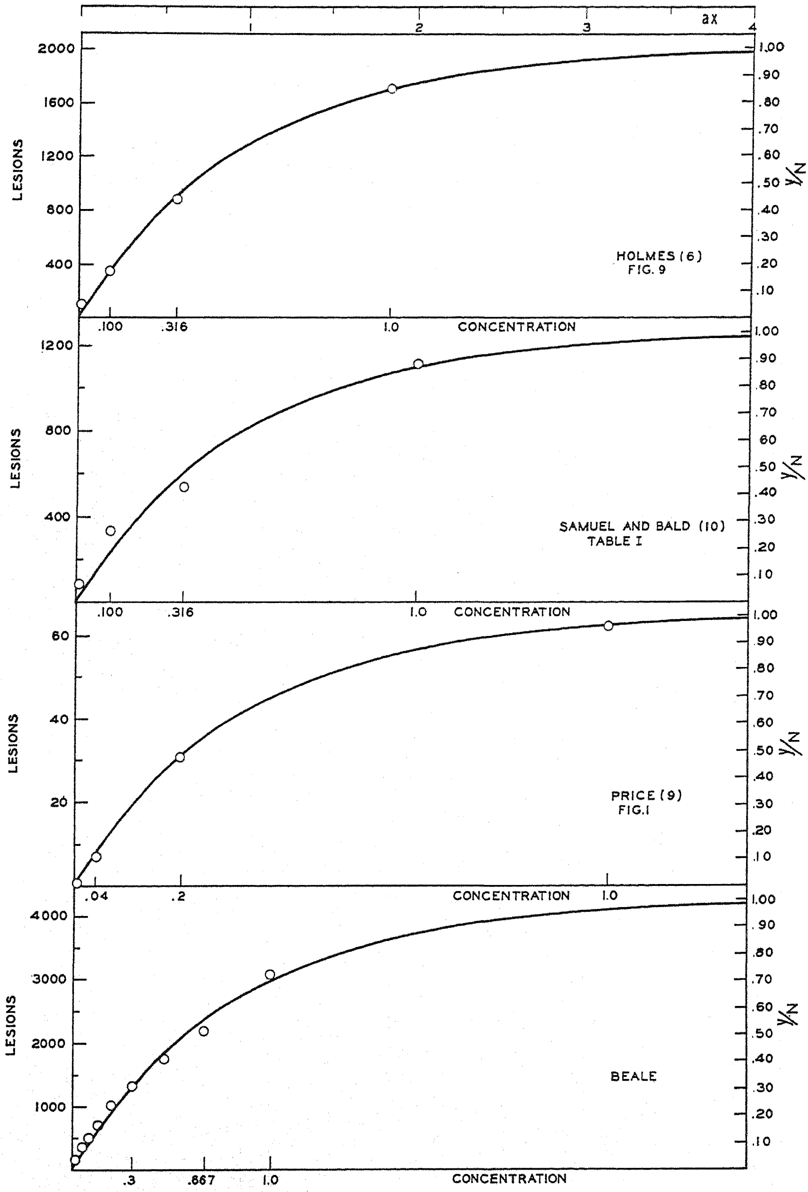

FIGURE 3. Curves for the same data used in Figure 2. All curves have the same ax scale shown at the top of the figure. The y/N scale of ordinates is the same for the four curves. The point marked 1.0 on each abscissa is plotted on the ax scale at the value obtained for a for each set of data. The figure shows that the curves could be superimposed upon each other.

mate. If these values are used as a basis for a new estimate, and the computations shown in Table I carried through, small additional corrections would be found which would slightly change the quantities listed. Any such changes would improve the agreement between the data and calculated values. The alteration in the calculated counts, however, is very slight.

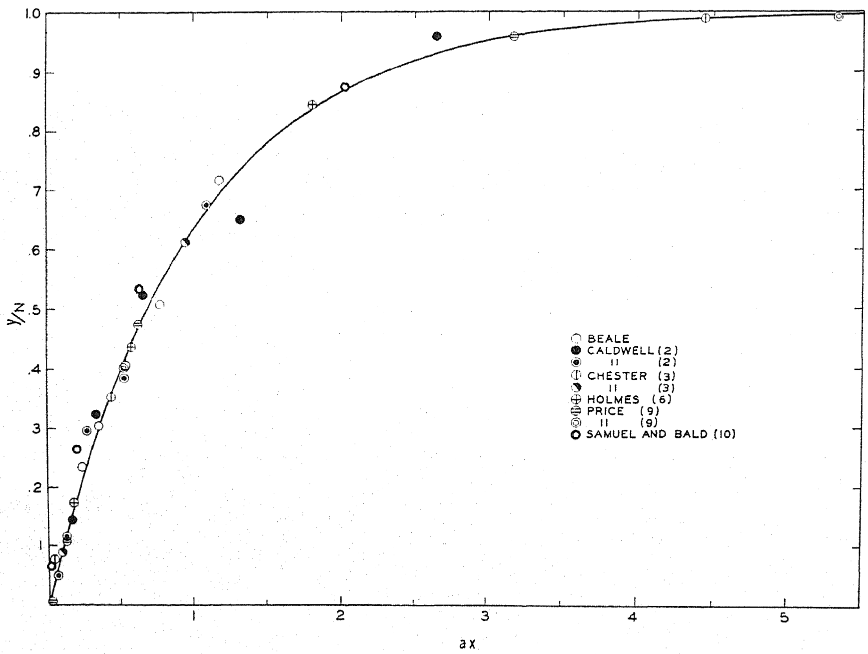

FIGURE 4. Data for nine dilution curves plotted on the same axes using the same procedure used for the curves shown in Figure 3. The figure shows how one standard curve may be used to represent various dilution experiments.

DISCUSSION

This compilation of the experimental results from several recent papers includes work done with tobacco mosaic, ring-spot, cucumber mosaic, and aucuba mosaic viruses using *Nicotiana glutinosa* L., *Phaseolus vulgaris* L. var. Early Golden Cluster, and *Vigna sinensis* (L.) Endl. var. Black Eye as test plants. Water, physiological saline, Ringer's solution, and tenth molar dibasic potassium phosphate (K_2HPO_4) have been employed as diluting agents. In some cases normal rabbit serum has been added to the virus extract. Some of the virus extracts were purified by basic and neutral lead acetate precipitations (13). They have been used either as soon as expressed from diseased plants or after being frozen and kept until needed.

In spite of the diversity of the material and procedure the same dilution law describes the results in a satisfactory manner.

If this equation is accepted tentatively as a close approximation of the relationship between virus concentration and lesion counts, it should serve as a guide to the design of new experiments as well as for the reinterpretation of work already reported. One disadvantage encountered in testing the equation is the choice of steps that have been employed in making the dilutions. If the undiluted preparation produces 1000 lesions then subsequent dilutions produce counts between zero and 1000. It would seem desirable to dilute in steps so that the range from zero to 1000 is divided into approximately equal intervals. The equation may be used as a guide in estimating the dilutions to use. More points in the middle range will facilitate the evaluation of the constants of the equation. The constant N may be considered as the maximum number of lesions that the leaf or plant can register. Samuel and Bald (10, p. 78), Beale (1, p. 424), and Chester (3, p. 1186) refer specifically to this maximum. The constant a appears to be a property of the virus preparation and is an efficient measure of its concentration. All the points (dilutions) contribute to the computation of a (and N) and thus it appears that a series of appropriate dilutions offers a means of accurately evaluating the concentration of the original material. Additional evidence regarding the meaning of the two constants will be afforded by experiments using the same virus on different lots of the same host. The lots should differ in age or other conditions which affect susceptibility. If the same a results from each experiment it is clear that a ready means will be at hand to conduct comparable tests over a long period of time regardless of changes in the test plants. The constant N may also be used to distinguish differences between various host plants. Similarly experiments with different virus extracts on the same host will disclose the adequacy of the constant a as a characteristic of the virus.

If a particular virus extract is so low in concentration that subsequent dilutions give counts proportional or nearly proportional to the dilution, the series is of no value in estimating the constant N. The inoculum must be concentrated enough to give some counts on the curved portion of the graph. It may be possible to use N determined on the same lot of plants using a more concentrated virus extract. The slope of the line in the dilute range is aN so that if N is known a may be easily obtained.

No attempt has been made to weigh the counts obtained at different dilutions. In some cases more plants were used at high dilutions so that the averages have been automatically given weights. In any event it is not difficult to modify the procedure for calculating the constants in accordance with the known precision at different parts of the dilution curve. It is also possible to compute the standard deviation of N and a when

TABLE II

TABULATIONS OF DATA AND CALCULATED CONSTANTS

Source	Conc.	Observed lesions	Calculated lesions	*N*	*a*	Test plant and virus	No. of plants or leaves
Holmes (6) Fig. 9	1.000 .316 .100 .010 .001	1700 880 350 100 40	1693 890 339 37 4	2012 (per plant)	1.85	*Nicotiana glutinosa* tobacco mosaic	10 plants
Price (9) Fig. 1	1.000 .200 .040 .008	62.1 30.8 7.0 0.5	62.14 30.45 7.72 1.62	64.86 (per leaf)	3.17	*Vigna sinensis* var. Black Eye, ring-spot virus	80 leaves
Price (8) Fig. 3	1.000 .100 .010 .001	870 420 287 41	863 464 64 7	863.4 (per leaf)	7.71	*Phaseolus vulgaris* var. Early Golden Cluster, tobacco mosaic	8 leaves
Price (8) Fig. 4	1.000 .100 .010 .001	1620 652 225 43	1629 675 85 9	1637 (per leaf)	5.31	*Phaseolus vulgaris* var. Scotia, tobacco mosaic	8 leaves
Samuel & Bald (10) Table I	1.0000 .3160 .1000 .0100 .0010 .0001	1104 534 332 86 8.8 0.7	1094 596 231 25 3 0.3	1260 (per plant)	2.03	*Nicotiana glutinosa*, tobacco mosaic	10 plants
Samuel & Bald (10) Table III	1.000 .316 .100 .0316 .010 .00316	12144 7470 4314 3363 2010 1003	11756 8572 3932 1415 467 150	11980 (total 20 half plants)	3.98	*Nicotiana glutinosa*, tobacco mosaic	10 plants
Beale*	1.000 .667 .466 .300 .200 .133 .090 .060 .040 .027	3067 2178 1732 1295 1010 692 486 350 215 125	2957 2326 1805 1272 902 620 430 292 197 134	4271 (total 48 half leaves)	1.18	*Nicotiana glutinosa*, tobacco mosaic	4 plants
Samuel & Bald (10) Fig. 3 Q 117	1.000 .316 .100 .0316 .010 .00316 .001 .00032	190 204 135 81 45 18 9 5	195 193 150 73 27 9 3 1	194.6	14.81	*Nicotiana glutinosa*, tobacco mosaic	—

* Obtained by using the Latin Square arrangement described by Youden and Beale (14).

TABLE II (*Continued*)

Source	Conc.	Observed lesions	Calculated lesions	N	a	Test plant and virus	No. of plants or leaves
Chester	1.0000	1220	1219.4	1223.4	5.72	*Phaseolus*	16–72
(3)	.1000	530	533.2	(per		*vulgaris* var.	leaves
Fig. 1	.0100	187	68.0	leaf)		Early Golden	
	.0010	25	7.0			Cluster,	
	.0001	2.9	0.7			tobacco mosaic	
Chester	1.0000	640	633.4	633.5	9.2	*Phaseolus*	16–72
(3)	.1000	350	381.1	(per		*vulgaris* var.	leaves
Fig. 3	.0100	195	55.7	leaf)		Early Golden	
	.0010	12	5.8			Cluster,	
	.0001	1.2	0.6			tobacco mosaic	
Chester	1.0000	430	429.7	692	0.97	*Nicotiana*	16–72
(3)	.1000	62	63.9	(per		*glutinosa*,	leaves
Fig. 5 G	.0100	20	6.7	leaf)		tobacco mosaic	
	.0033	13.9	2.2			+	
	.0010	4.7	0.7			normal serum	
	.0003	1.1	0.2				
Chester	1.0000	1090	1089.8	1103	4.42	*Phaseolus*	16–72
(3)	.1000	388	394.3	(per		*vulgaris* var.	leaves
Fig. 5 B	.0100	87	47.6	leaf)		Early Golden	
	.0033	84.7	16.1			Cluster,	
	.0010	22.3	4.9			tobacco mosaic	
	.0003	5.2	1.7			+	
						normal serum	
Chester	1.0000	1180	1180	1181	6.93	Tobacco mosaic	16–72
(3)	.1000	590	590	(per		lead purified	leaves
Fig. 6	.0333	290	244	leaf)			
	.0100	79	79				
	.0033	73	27				
	.0010	15	8				
	.0003	5.8	3				
	.0001	2.0	1				
Chester	1.000	4.7	4.85	4.87	5.65	*Vigna sinensis*	20
(3)	.333	4.4	4.13	(per		var.	leaves
Fig. 9	.100	2.1	2.10	leaf)		Black Eye,	
	.033	0.49	0.84			cucumber mosaic	30–80
	.010	0.06	0.27				leaves
Caldwell	1.0000	1610	1556	1676	2.64	*Nicotiana*	—
(2)	.5000	1090	1228			*glutinosa*,	
p. 106	.2500	882	810			aucuba or	
	.1250	540	471			yellow mosaic	
	.0625	245	255			of tomato	
Caldwell	1.0000	530	523	785.7	1.10	*Nicotiana*	10
(2)	.5000	300	331	(total		*glutinosa*,	leaves
Table III	.2500	230	188	10		aucuba mosaic	
	.1250	90	100	leaves)			
	.0625	40	52				
	.03125	50	26				
	.01563	11	13				
	.00781	3	7				

these constants are computed from lesion counts made at a series of dilutions.

The experiments by Caldwell (2) using known volumes of virus extracts on measured areas of host should be continued. Suitable data of this type coupled with the relation between counts and dilution may make possible more accurate estimates of the number of particles in the inoculum. Caldwell (2, p. 114) reports that a given volume of virus extract whether placed on half a leaf or a whole leaf gave the same number of lesions. It is clear that in the case of the half leaf there were twice as many particles per susceptible area as in the case of the whole leaf. In the dilute range this doubles the count per half leaf and consequently as many lesions were obtained as on the whole leaf. The area does take on importance if concentrated preparations are employed.

Several dilution curves have been taken from a paper by Chester (3). The curves were incidental to some experiments designed to differentiate between the effect of normal serum and antiserum on virus infectivity. Chester concludes that the normal serum lowers the lesion count through an effect on the host plant while the antiserum reacts on the virus as well. In terms of the equation discussed in this paper the normal serum reduces the value of N, the antiserum of both N and a. The first example given by Chester shows a loss of $\frac{1}{3}$ in the number of lesions upon addition of serum to the virus extract. He further states that the maximum number of lesions is close to the count (1220) obtained with his most concentrated extract. Hence N is either reduced to $\frac{2}{3}N$ or the virus has been inactivated to such an extent that the lower concentration infects but $\frac{2}{3}$ the available areas. The upper half of Figure 5 shows the dilution curve of the virus plotted from constants calculated from his data, as well as two derived curves, one on the assumption that N is reduced to $\frac{2}{3}$ its value and the other assuming that a is reduced. The curve for virus alone shows that an extract of $\frac{1}{5}$ the original concentration will give about 800 lesions or $\frac{2}{3}$ the original count. This means that $\frac{4}{5}$ of the virus is no longer active. The curves show the lesion counts that would be expected at various dilutions assuming first, that N has been reduced to $\frac{2}{3}N$, and second, that a constant fraction ($\frac{4}{5}$) of the virus is inactivated at the various dilutions. On the basis of an action on the host the count is always $\frac{2}{3}$ that of the untreated virus. The curve postulating an effect on the virus shows the count diminishes more rapidly with the dilution. It reaches a constant fraction at about a 10- to 30-fold dilution. The curves have been replotted in the lower half of Figure 5 according to the scheme adopted by Chester. The similarity of the curve, assuming an effect on the virus, to the experimental ones shown in Figures 1 to 4 in Chester's paper is immediately apparent. The application of the equation leads to the conclusion that the normal serum reacts on the virus.

This concept of a maximum number of lesions must in some way be reconciled with the observation by Samuel and Bald (10) and others of the effect of sand in increasing the number of lesions. It may be that N is a

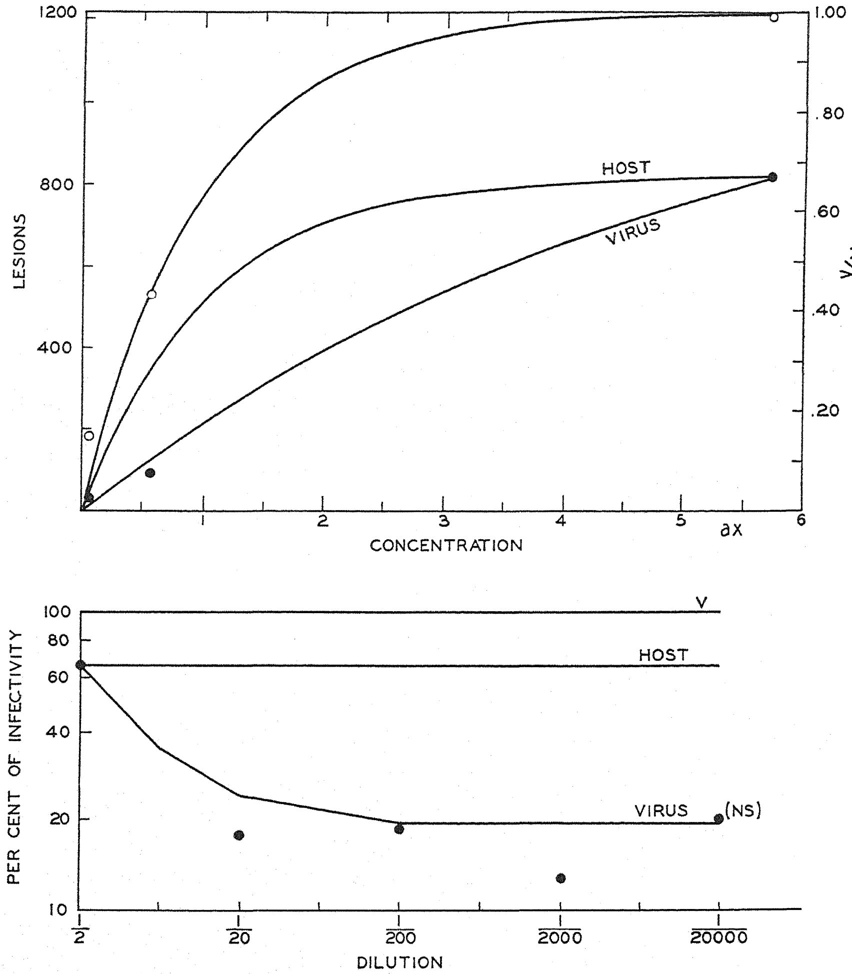

FIGURE 5. Data taken from Chester (3) Figure 1 have been plotted in the upper half of the figure. The uppermost curve has been drawn using constants calculated from his data. The curve marked "host" has been drawn assuming N to be $\frac{2}{3}$ the previous value. The lower curve marked "virus" has been drawn assuming a to be $\frac{1}{5}$ the previous value. The black dots represent experimental values obtained with virus plus normal serum. The lower half shows the same curves replotted according to the method used by Chester. Due to the logarithmic scale all the highest dilutions appear. (These would be crowded in the left-hand corner of the upper chart.) The experimental points for virus plus serum lie along the line drawn on the assumption of a smaller a.

constant for any particular technique employed. Another point of considerable interest is the behavior at high dilutions. On the basis of the equation the predicted counts at extremely high dilutions are usually below those actually found. Possibly at high dilutions aggregates of particles are broken up which in high concentrations act, through their proximity, as one particle. Kunkel in a brief abstract (7) mentions experiments with mixed viruses that showed that lesions seldom contained both kinds. Data of this type will afford an excellent opportunity to check computations of the frequency of lesions resulting from two particles.

SUMMARY

Data from six different sources showing lesion counts obtained by applying virus extracts to leaves in a series of dilutions have been assembled from the literature. The curves include work with tobacco mosaic, ring-spot, cucumber mosaic, and aucuba mosaic viruses. The plants used as hosts have been *Nicotiana glutinosa*, *Phaseolus vulgaris* var. Early Golden Cluster, and *Vigna sinensis* var. Black Eye. All the dilution curves have been satisfactorily fitted using the relation

$$y = N(1 - e^{-ax})$$

where y is the lesion count and x the concentration. The method of evaluating the constants is given and their possible significance discussed. The utility of the function as a guide to the design and interpretation of experiments is shown by examples.

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THIOUREA PREVENTS BROWNING OF PLANT TISSUES AND JUICES[1]

F. E. Denny

The freshly-cut surfaces of many (but by no means all) kinds of plant tissue turn brown rapidly, and this oxidation can be prevented by exposing the tissue to sulphur dioxide. Overholser and Cruess (5) made a special study of apple tissue in this connection and found that SO_2 prevents the darkening of apple tissue by an effect upon the organic peroxide in the tissue, rather than by influencing either the oxidizing enzymes or the chromogen from which a colored compound is formed upon oxidation. They found a number of other chemicals which retarded browning, especially NaCl which at a concentration of 3 to 5 per cent had a distinctly favorable effect and in addition reduced the amount of SO_2 needed to inhibit browning.

Recently Balls and Hale (1, 6) report that glutathione or cysteine salts if applied to sliced apples permit drying without discoloration. They ascribe this effect to an influence of the sulphydryl compound upon the enzyme peroxidase. As a source of the sulphydryl compound they used pineapple juice. Sliced apples when sprayed with pineapple juice either as freshly-expressed or after fermentation were dehydrated without darkening, and remained white after many months of storage.

The object of the present paper is to point out that a solution made by dissolving one gram or more of thiourea in a liter of water (approx. 0.1 per cent) is very effective in preventing browning of slices of apples and certain other plant tissues. When added to freshly-expressed apple juice, about 0.1 gram of thiourea per liter of juice (approx. 0.01 per cent) is sufficient to prevent browning, and, indeed, to decolorize juice which has already become brown by a few minutes exposure to air.

It should be stated at once that no recommendation is made as to the use of thiourea in connection with a food product. Very little experimental evidence as to the toxicity of thiourea is available at present, and, while the data indicate that it is low in toxicity, or possibly even non-toxic, much more information must be obtained before a conclusion can be formed upon a question as important as this one. At this time, emphasis is placed merely upon the effectiveness of thiourea in preventing darkening of tissue and juice.

There was evidence of this action of thiourea in previous experiments with the use of chemicals in hastening sprouting of dormant potato tubers. Cut tubers when soaked in thiourea solutions and planted in soil did not

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 87.

develop a dark-brown color but remained for many days in the soil with only a slight development of brown color. The self-reducing capacity of juices obtained from tubers which had been treated with thiourea has been referred to in a previous article (2, p. 497), and the unusually strong inhibiting effect of thiourea and other sulphur compounds on the tyrosinase action of potato juice has been pointed out (3, p. 74). It was because of these previous observations on the effect of thiourea upon the coloration of potato tissue and juice that the present experiments with apple and other tissues were undertaken.

EXPERIMENTS WITH SLICES OF TISSUE

The apple (*Pyrus malus* L.) fruits were peeled, cut into eighths, placed in beakers, covered with the thiourea solution, and allowed to remain for one minute. The slices were removed, arranged on filter papers, and allowed to dry. In some experiments the drying occurred in still air at room temperature, in others in a current of air, in others at 35° C. in a current of air, and in others in still air at 50° C. Lighter colored products were obtained under conditions favoring rapid drying. Thiourea solutions containing 30, 10, 3.33, 2, and 1 g. per l. were effective in preventing darkening of the fruit. At concentrations of 0.5, 0.1, and 0.01 g. darkening was retarded but subsequently some color developed. For most of the tests the variety McIntosh was used but confirmatory results were obtained with Baldwin, Greening, Northern Spy, and Stayman Winesap. Lots A and B in Figure 1 show the effect or thiourea in preventing browning of slices of McIntosh apple.

Other tissues tested were pear (*Pyrus communis* L.), banana (*Musa sapientum* Kuntze.), eggplant (*Solanum melongena* L.), and potato (*Solanum tuberosum* L.). Successful results were obtained with the first three but potato tissue, although its coloration was retarded, subsequently became black. Coloration of the potato could be prevented only if the tissue was kept in a moist condition.

Simultaneously with these tests with thiourea, slices of tissue were soaked for one minute in 3 per cent NaCl and in pineapple juice, and were dried under the same conditions. Confirmation of the reports of Overholser and Cruess (5) for NaCl, and of those of Balls and Hale (1) for pineapple juice was obtained. However, in the case of eggplant tissue the results with thiourea were distinctly better than with either NaCl or pineapple juice.

In most of the tests the tissue was immersed in the thiourea solution for one minute. Subsequent experiments with McIntosh and Northern Spy apples indicated that merely dipping the slices into the thiourea solution and removing at once was effective and gave the same protection as that obtained by the longer period of contact.

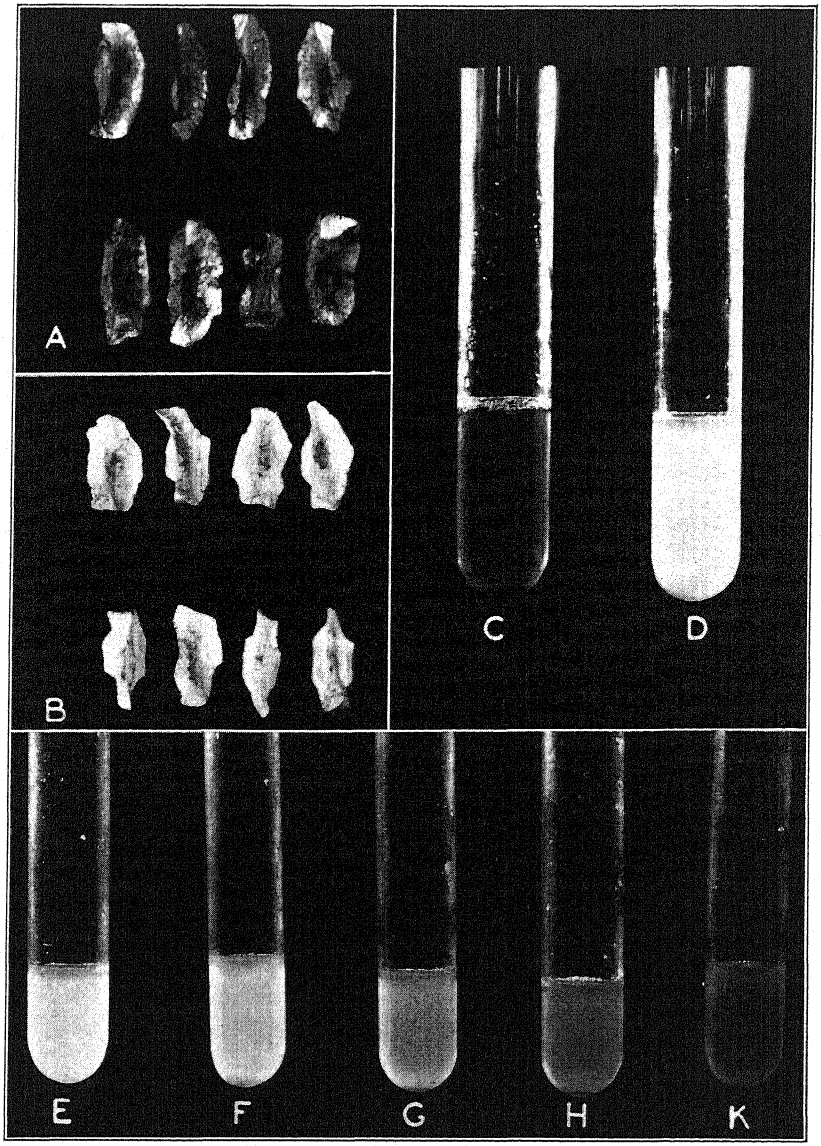

FIGURE 1. A. Slices of McIntosh apple allowed to dry in air; B. Slices of McIntosh appled soaked one minute in 0.1 per cent thiourea solution and allowed to dry in air; C.Brown apple juice, check lot; D. Brown apple juice decolorized by adding 1 mg. of thiourea per 10 cc. of juice; E. Thiourea added to juice at once after pressing; F. After being exposed to oxidation in air for 15 minutes before adding thiourea; G. For one hour; H. For two hours; K. For four hours. Residual liquid after decolorizing action of thiourea shows increasing amounts of an unreducible pigment as duration of the preliminary oxidation was increased.

When apple slices which had been treated with thiourea and which had partly dried without browning were soaked for one minute in water and were again placed under drying conditions browning began promptly. This showed that thiourea had to be present to prevent browning, and that it had not destroyed or rendered permanently inactive any constituent that was essential for browning.

The mode of action of thiourea in preventing browning was tested from the point of view developed by Overholser and Cruess (5). They described a system of tests to determine whether the effect of a treatment was upon the organic peroxide or upon the peroxidase. Development of a blue color when the slices were treated with benzidine solution indicated that both enzyme and organic peroxide were present. If the blue color did not develop until after the addition of hydrogen peroxide, peroxidase was active but the organic peroxide did not function.

The results of the application of such tests to apple slices with and without treatment by thiourea are shown in Table I. They indicate that the thiourea effect was principally upon the organic peroxide and not upon the peroxidase since the blue color did not develop until after hydrogen peroxide was added. It is true that the blue color resulting from adding H_2O_2 and benzidine to the thiourea-treated slices was less intense than that obtained with the check tissue, which suggests the possibility that the peroxidase activity may have been lowered somewhat by the thiourea, as has been found to be the case with potato peroxidase (2, p. 493).

TABLE I

TESTS OF ACTION OF THIOUREA UPON THE OXIDASE SYSTEM OF APPLE TISSUE

Reagent applied to apple slices	Resulting color	
	Slices not previously treated	Slices previously treated with 0.1% thiourea
Nothing	Brown	White
H_2O_2	Brown	White
Benzidine	Bluish	White
Benzidine+H_2O_2	Dark blue	Blue

Overholser and Cruess (5) also tested directly for organic peroxide by treating the slices with a solution of potassium iodide containing starch. In the presence of peroxide iodine is liberated from the KI and gives a blue color with the starch. This test could not be used conclusively in the present experiments with thiourea since this reagent reacts with iodine. Hence the negative result which was obtained with thiourea-treated apple slices could have occurred even in the presence of organic peroxide, the iodine reacting with the thiourea rather than with the starch.

EXPERIMENTS WITH APPLE JUICE

Apple tissue which is passed through a food grinder and pressed yields a juice which is already brown due to oxidation during grinding and squeezing. The addition of thiourea to the brown juice obtained in this way caused the juice to decolorize in a few minutes to a nearly colorless juice (see Fig. 1 C and D), and this juice did not darken upon subsequent standing for several hours. By changing the method of extracting juice it was found possible to obtain a juice with practically no brown color. This was done by cutting the tissue into small cubes, wrapping them in cheesecloth, squeezing in a small press, and centrifuging the juice. Addition of varying amounts of thiourea to such juice showed that about 1 mg. of thiourea per 10 cc. of juice was sufficient to prevent the development of the brown color. The rate of development of color was retarded by amounts as low as 0.2 mg. per 10 cc. of juice.

When the freshly-expressed juice was poured into large watch-glasses in order to obtain a thin layer, and was exposed to air for varying periods before the thiourea was added, thiourea in amounts of 1 to 5 mg. per 10 cc. of juice produced a marked decolorization of all lots whether the period of exposure to air had been short or long. However, the color of the liquid which was obtained after the thiourea had carried the decolorization process as far as it could, depended upon the time after pressing before the thiourea was added. The longer the juice stood in air the darker the final liquid. Tubes E to K in Figure 1 show such a result. At the 15-minute period the juice, although it was quite brown at the time of the addition of thiourea, was practically completely reduced and the resulting liquid was nearly colorless. However, the juice taken at later periods was not completely reduced and the final liquid always was pigmented, the color of the residual liquid increasing as the time periods of preliminary oxidation increased. Tubes E to K in Figure 1 show how the amount of this pigment increased with time, requiring about four hours to reach a maximum.

Experiments with pineapple juice helped to explain these rather confusing results with thiourea. Pineapple juice was added to apple juice at varying stages of oxidation as was done with thiourea. A volume of pineapple juice equal to one-fifth of the volume of the apple juice produced a decolorization of brown apple juice in a manner similar to that obtained with thiourea, but with the following very important difference. For a few minutes after the addition of the pineapple juice the reduced color was maintained at a level about equal to that obtained with thiourea, but upon subsequent standing the liquid in the tubes containing pineapple juice began to develop a brown color which increased in intensity for an hour or two. Thus, rebrowning occurred in the presence of pineapple juice but not in the presence of thiourea.

The causes of this rather complicated behavior are not known, but an explanation which seems to reconcile all of the observations is obtained if we assume that there are two different chromogens and two different reactions which proceed as follows:

Reaction I	Chromogen A	$\xleftrightarrow[\text{Both thiourea and pineapple juice are effective}]{\text{Reversible, rapid}}$	Dark-brown oxidation product
Reaction II	Chromogen B	$\xrightarrow[\text{Thiourea inhibits but pineapple juice does not}]{\text{Not reversible, slow}}$	Light-brown oxidation product

Both thiourea and pineapple juice were able to reduce the dark-brown product in Reaction I to give relatively colorless compounds; but if Reaction II has already proceeded partially at the time of adding the reagent, thiourea could not reduce the oxidation-product already formed, although it could prevent any further oxidation of chromogen B. Pineapple juice could neither reduce the oxidation product of chromogen B nor stop its further oxidation. Hence, with pineapple juice the preliminary decrease in color due to reduction of the oxidation product in Reaction I was followed by a subsequent increase in color due to the oxidation in Reaction II.

Admittedly these assumptions cannot be considered conclusive until chemical evidence as to the identity of these two chromogens, their chemical characteristics, and oxidation products is obtained, but at least they furnish a basis for further tests.

TOXICITY OF THIOUREA

Only two references have been obtained at present regarding the toxicity of thiourea. Wurtz (7, p. 126) says: "La sulfo-urée, ingérée même à assez haute dose, n'exerce sur l'économie aucune influence appréciable." But the experimental evidence upon which this statement is based is not disclosed, nor are the citations given.

Nicolas and Lebduska (4) experimenting with dogs and rabbits found that the intravenous injection of 10 to 11 g. of thiourea per kilogram of weight caused death. However, the introduction of 1 g. per kg. of weight into the duodenum of the dog did not increase the blood pressure nor influence the rate of respiration. They conclude that thiourea is less toxic than urea.

However, with so little information available as to its toxicity the use of thiourea with food products cannot be recommended at present.

SUMMARY

Soaking slices of apple tissue for one minute, or even by dipping, in a 0.1 per cent solution of thiourea prevented browning of the cut surfaces upon subsequent drying in air. Favorable results were obtained also with pear, banana, and eggplant. With potato retardation but not inhibition of darkening resulted.

The addition to freshly-expressed apple juice of thiourea to give a concentration of 0.01 per cent prevented darkening. And if the juice had already become brown by contact with air this amount of thiourea induced decolorization, to a colorless condition if added soon after pressing and to a much lighter brown color if the oxidation had proceeded in air for as much as four hours.

The addition of one cc. of freshly-expressed pineapple juice to four cc. of brown apple juice also caused decolorization, but subsequently re-browning occurred.

Evidence was obtained that in the coloration of apple juice by oxidation in air there are two reactions occurring simultaneously: one being rapid and reversible, forming a dark-brown oxidation product which can be reduced to relatively colorless compounds by the addition of either thiourea or pineapple juice; the other being slow, not reversible, forming a light-brown oxidation product, the oxidation of which is inhibited by thiourea but not by pineapple juice, and the reduction of which cannot be carried out by the addition of either thiourea of pineapple juice.

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A STUDY OF SOME FATTY ACIDS AND THEIR SOAPS AS CONTACT INSECTICIDES[1]

L. E. DILLS[2] AND H. MENUSAN, JR.[3]

Soap is one of the oldest and most universal constituents of contact insecticides and has been widely used in combating sucking insects for at least a century. Besides possessing considerable toxicity alone, it is often used as a spreader and wetter for other spray materials and as an emulsifier for petroleum oils. An examination of the literature of insecticides shows that there are several thousand references on the use of soaps. As a high percentage of these references quote experimental data it would seem that there should be an abundance of information upon their insecticidal value, but a more careful examination of the whole problem discloses a confusing situation. Practically all experiments have been conducted with soaps of an unknown water content. A number of soaps commonly used in sprays were examined (during this work in New York State) and the water content was found to vary from 30 to 70 per cent by weight in different brands. Since the average economic entomologist rarely determines the moisture content of the materials tested it is easy to see how even carefully conducted tests could give highly variable and erroneous results.

Another cause of variable results, and one not so easily corrected, is the variation in the proportion of the various fatty acid salts. If these are not all equally toxic to insects, obviously any change in proportion would affect the insecticidal value of the material.

Most of the experiments have been conducted by research workers primarily interested in answering the agricultural problems in a particular community. Usually they compare different commercial brands instead of different kinds of soap and their conclusions and recommendations are reliable only until the manufacturer changes his product. For that reason most of the experiments in the use of soaps have only transient value and it is found necessary to repeat them each time a new brand appears or an old one is altered. An enormous amount of money, time, and labor has been expended in determining the toxicity of different brands of soap and it seems highly desirable that the toxicity of the various constituents of soap be examined and their relative value determined. It was from this point of view that the experiments reported at this time were conducted.

[1] This study gives the results of certain aspects of a cooperative project carried on at the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, with funds supplied by the Research Corporation, New York, New York.

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Siegler and Popenoe (9, 10) suggest the hypothesis that the toxic action of soap is due to the free fatty acids which are liberated upon hydrolysis. Working on this assumption they tested various impure distillates of emulsions of the fatty acids and conclude that the peak of toxicity for the saturated fatty acids is near capric acid.

Tattersfield and Gimingham (11) of the Rothamsted Experimental Station several years later conducted tests with fatty acids. They used 16 acids in the saturated series and two unsaturated acids in different concentrations. Their experiments were performed with only ten aphids in a test, but their work seems to have been accurately conducted and apparently gives good approximations. After testing the acids alone the same acids were made into sodium and ammonium salts and methyl esters and the toxicity tests were repeated in order to determine the toxicity of the hydrocarbon chain alone.

McGovran (7) and Filmer (1) found that sodium oleate with nicotine is more toxic to aphids than is fish oil soap and an equal amount of nicotine. These materials were used on a dry weight basis and enough tests were made to give reliable results.

Using adults of the Japanese beetle as experimental insects van der Meulen and Van Leeuwen (12) tested the sodium and potassium soaps made from 20 different vegetable and animal oils. Their results show that soaps made from oils or fats from different sources have a remarkable difference in toxicity.

MATERIALS

The purest caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic acids obtainable were procured from Eastman Kodak Company and from Eimer and Amend. Their purity was checked by the determination of their saponification number (milligrams of potassium hydroxide required to neutralize a gram of acid) which in every case approximated the calculated theoretical values. The oleic acid was guaranteed to be free from linoleic acid.

The potassium soaps were made by treating weighed portions of the pure acids with the calculated amount of potassium hydroxide, then refluxed for a period of two hours to promote neutralization, especially with the acids of high molecular weights. When saponification was complete, the solution of potassium soap was diluted to 10 or 20 per cent by volume of actual soap. The stearate and palmitate soaps gave a gel which rendered it necessary to dilute to 5 per cent (by volume) in order that they remain liquid enough to measure at room temperature. At the time of spraying, these stock soap solutions were diluted with distilled water to the desired concentration.

The nicotine sulphate was made by carefully neutralizing pure nicotine with dilute sulphuric acid with methyl red as an indicator.

METHOD

The principal test insect used in the laboratory to measure the relative toxicity of contact insecticides was the bean aphid (*Aphis rumicis* L.). This aphid was reared on dwarf nasturtium plants (*Tropaeolum minus* L.) grown in the greenhouse, or in the open during favorable weather.

The material to be tested, in water solution or suspension, was sprayed on the infested plants and counts were made following a method described by Hartzell and Wilcoxon (5).

Tests in which the rose aphid (*Macrosiphum rosae* L.) was used as the test insect were conducted in a similar manner, with the exception that immediately before spraying, infested stems were taken from the rose garden and their cut ends placed in Erlenmeyer flasks filled with water.

Preliminary tests indicated that the youngest stages of the bean aphid were relatively easier to kill than the adult. This is in agreement with the generally accepted idea that immature insects are easier to kill. Hartzell and Wilcoxon (4) found that the immature stages of the red spider mite (*Tetranychus telarius* L.) were less resistant than the adult to naphthalene fumigation.

To measure the resistance of the developmental stages of the bean aphid to contact insecticides, a number of infested plants were sprayed with various concentrations of sodium oleate ranging from one-eighth to three-fourths of 1 per cent. The number killed by this treatment varied from 20 to 95 per cent, and in each case a higher mortality was obtained with the younger nymphs. A summary of the results obtained is given in Table I.

TABLE I

RESISTANCE OF THE DEVELOPMENTAL STAGES OF THE BEAN APHID

Developmental stage	Number counted	Number dead	Per cent dead
First instar	1151	749	65.1
Second instar	307	229	74.6
Third instar	284	162	57.0
Fourth instar	105	48	45.7
Adult	136	68	50.0
Totals	1983	1256	63.3

The mortality of the water checks during the above experiments counting 684 insects was 3.6 per cent. It will be noticed that the first instar is apparently more resistant than the second instar; this anomaly is due to the fact that the living adults, viviparous females, were giving birth to young during the experiment. Using Fisher's Chi-square test for homogeneity (2, p. 178–237), it is possible to show that the differences between the different instars are greater than those which might arise through

chance alone with the exception of adults and the fourth instar, which are not significantly different.

RESULTS OF INSECT TESTS

WITH FATTY ACIDS

The fatty acids are relatively insoluble in water and most of those used are solid at room temperature. For comparative toxicity tests these acids were finely dispersed in water. The liquid acids caproic, caprylic, and oleic formed an emulsion and all the others a suspension of more or less solid particles. To obtain highly dispersed systems it was found advisable to melt the fatty acids with the emulsifier, then to add the required amount of hot distilled water. In the liquid fatty acids this procedure was not necessary because the acid, emulsifier, and water could be mixed together without heating. The dispersions obtained in this manner are stable and can be kept for months without apparent segregation. In toxicity determinations fresh dispersions were always made just before spraying.

Preliminary trials indicated that a sulphonated cod oil ("Tanoyl 990" manufactured by the National Oil Products Company) was a much better emulsifier than saponin. Thereafter, this sulphonated oil was used throughout the experiments as an emulsifier for the fatty acids. This substance was only slightly toxic to the bean aphid. The results obtained with saponin and sulphonated cod oil are given in Table II.

TABLE II

TOXICITY OF EMULSIFIERS TO APHIS RUMICIS

Emulsifier	Per cent conc.	Total No. of insects counted	Per cent dead
Sulphonated cod oil	0.25	452	4.0
Sulphonated cod oil	0.50	512	9.0
Saponin	0.25	497	3.0
Saponin	0.50	535	3.7
Check (water)	—	371	1.3

It has been previously found that when the mortality of the check exceeded 10 per cent, correcting by means of the formula $K = \frac{(T-C)\times 100}{100-C}$, did not always give results that agreed with tests conducted when the mortality of the check was low. In the formula T and C are the per cent dead in the treated and check respectively, while K is the corrected kill in per cent.

In the experimental results no attempt has been made to correct for the mortality of the check nor for the slight toxicity of the emulsifier. In

cases where the mortality of the check exceeded 5 per cent the experiments were discarded.

Toxicity experiments were repeated four times in an outdoor insectary with the following fatty acids: caproic, capric, lauric, palmitic, stearic, and oleic. In each case one-sixth of 1 per cent of fatty acid was used with one-quarter of 1 per cent of sulphonated cod oil as the emulsifier. On the fourth trial caprylic and myristic acids were tested in addition to the six previously listed. The temperature varied from 20° to 25° C. with a mean of about 22° C. while the relative humidity varied between 40 and 50 per cent. A summary of the results is given in Table III.

Excluding the acids caprylic and myristic and the emulsifier because of only one determination of their toxicity, one is able to analyze the experimental results obtained statistically by the variance method (2, 14). The standard error of a single toxicity observation is found to be 4.5 per cent and the standard error of the difference between means is 3.2 per cent. Differences equal to or greater than 2.15 times the standard error of mean differences are considered significant. This gives odds of 30 to 1 that the differences are not due to chance. Therefore, all differences between means in Table III equal or greater than 6.9 per cent are probably significant.

TABLE III

TOXICITY OF THE FATTY ACIDS TO APHIS RUMICIS

Emulsifier 1/4% plus acid 1/6%	No. of carbon atoms	No. of tests	Total No. of insects used	Mean % dead
Caproic	6	4	1510	15.4
Caprylic	8	1	534	18.1
Capric	10	4	2058	45.0
Lauric	12	4	1758	42.0
Myristic	14	1	527	16.9
Palmitic	16	4	2228	15.1
Stearic	18	4	2080	10.8
Oleic	18	4	1700	19.3
Check (water)	—	4	1405	2.7
Emulsifier (alone)	—	1	452	4.0

The tests indicate that capric and lauric acids are more toxic to the bean aphid than oleic, caprylic, myristic, caproic, and palmitic acids while stearic is the least toxic of the fatty acids tested. Stearic acid, however, is not significantly less toxic than caproic or palmitic acids but is less toxic than oleic acid. The peak of toxicity of the saturated fatty acids agrees with the tests reported by Tattersfield and Gimingham (11) and the conclusions reached by Siegler and Popenoe (9, 10).

It is interesting to note that in a test conducted at a high temperature (32° C.) there was a considerably higher per cent of bean aphids killed. This was particularly noticeable with the more toxic fatty acids; however,

there was no tendency for the relative order of the efficiency of the materials to be reversed.

With the rose aphid the order of toxicity of the fatty acids is the same as with the bean aphid.

WITH SOAPS OF THE FATTY ACIDS

The method of making the soaps and their application in the form of a spray has been previously described. The only difference in the soap tests with the bean aphid is that immediately after spraying, the plants were placed in a constant temperature room. The room was kept at 22° C.

TABLE IV

TOXICITY OF POTASSIUM SOAPS TO APHIS RUMICIS AND MACROSIPHUM ROSAE

Soaps 0.5% conc.	No. of carbon atoms	*Aphis rumicis*			*Macrosiphum rosae*		
		No. of tests	Total No. of insects	Mean % dead	No. of tests	No. of insects	% dead
Caproate	6	3	1803	6.9	1	139	3.6
Caprylate	8	1	144	36.9	—	—	—
Caprate	10	3	1769	55.2	1	189	19.0
Laurate	12	3	1910	67.1	1	327	61.1
Myristate	14	1	200	30.4	—	—	—
Palmitate	16	3	2257	24.9	1	360	11.1
Stearate	18	3	1995	12.8	1	226	3.1
Oleate	18	3	2092	85.1	1	235	88.5
Check (water)	—	3	925	4.9	1	206	2.4

with a relative humidity of 75 per cent. With the rose aphid the test was conducted in an outside insectary where the temperature varied between 20° and 24° C. and the relative humidity was approximately 45 per cent. The results obtained are summarized in Table IV.

TABLE V

TOXICITY OF OLEATES TO APHIS RUMICIS

Conc. of soaps in %	Potassium oleate				Sodium oleate			
	Test A		Test B		Test A		Test B	
	No. of insects	% dead	No. of insects	% dead	No. of insects	% dead	No. of insects	% dead
1.00	587	97.9	509	97.6	—	—	—	—
0.75	527	96.1	467	94.8	—	—	—	—
0.50	434	83.2	256	81.2	263	83.6	370	82.2
0.25	623	44.3	407	32.2	266	60.1	1032	53.0
0.25	—	—	749	41.5	—	—	—	—
0.125	817	10.5	898	14.5	224	22.2	1164	23.9
Check	—	—	403	5.2	688	3.6	—	—

Excluding caprylate and myristate as before, statistical analysis can be applied to the results obtained with the bean aphid. Differences between means greater than 8.5 per cent are found to be significant.

With the bean aphid the order of toxicity of the potassium soaps at one-half per cent concentration was found to be: oleate, laurate, caprate followed by the equally toxic caprylate, myristate, and palmitate which are more toxic than the apparently non-toxic caproate and stearate. Similar results (Table IV and Fig. 5) were obtained with the rose aphid. The

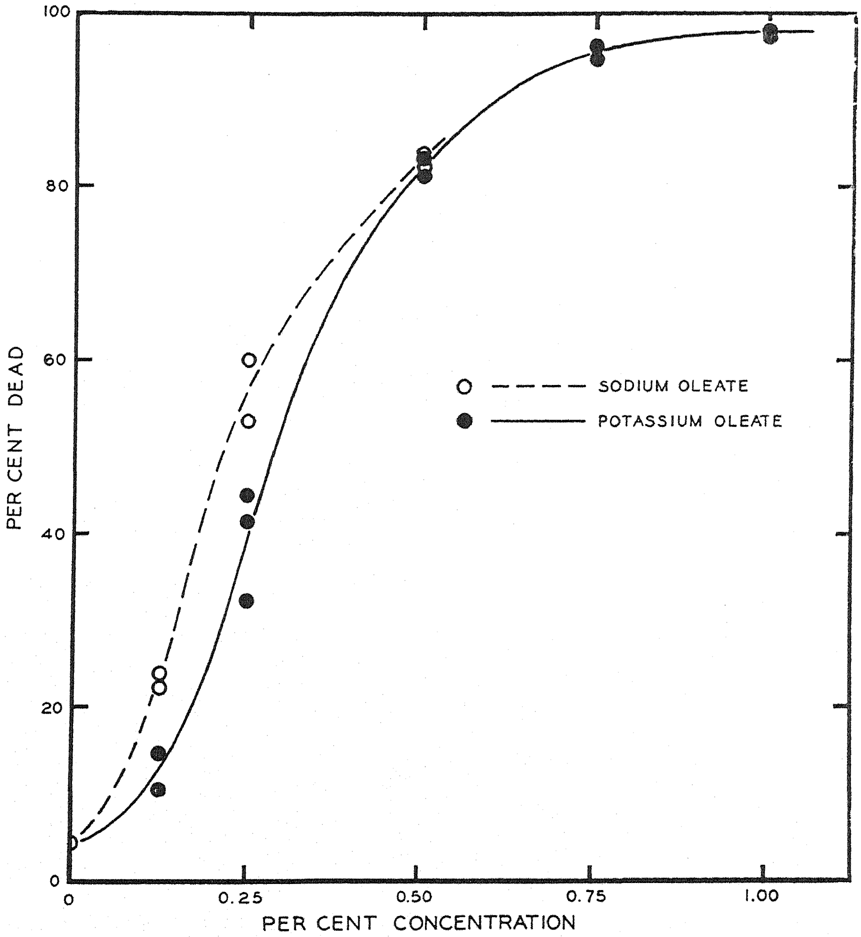

FIGURE 1. Comparative toxicity of sodium oleate and potassium oleate to *Aphis rumicis*.

order of soap toxicity, it will be noticed, is somewhat different from that obtained with the fatty acids at one-sixth per cent concentration; though in general the more toxic acids gave the more toxic soaps.

The oleate, the most toxic of the soaps tested, was used to determine what effect concentration would have on the toxicity to the bean aphid. For this determination sodium oleate and potassium oleate were both used.

The results obtained are reported in Table V and plotted in Figure 1. The concentration-toxicity curve is a definite S-shaped curve. Between 20 and 60 per cent mortality there is an approximately linear relationship between the toxicity of the soaps and their concentration. The sodium oleate, especially at low concentration, is apparently more toxic than the potassium oleate; this is probably due to the difference in molecular weights of the alkali used. When the concentration is expressed molecularly, the toxicity curves of sodium and potassium oleates coincide.

WITH SOAPS AND NICOTINE SULPHATE

Since soaps are seldom used alone as insecticides but are usually used in combination with nicotine sulphate or other contact insecticides, it appeared desirable to repeat the toxicity experiments with a reduced concentration of soap and a small amount of nicotine sulphate.

These experiments were first conducted in a 22° C. constant temperature room with the relative humidity at 75 per cent. The repetition of the experiments in an outside insectary where the temperature varied between 22° to 25° C. and the relative humidity between 40 and 45 per cent, gave a much lower mortality. The greater mortality obtained in a confined space may be due to the fumigating effect of nicotine. The results reported in Table VI are those obtained in the outside insectary.

TABLE VI

TOXICITY OF NICOTINE SULPHATE WITH DIFFERENT SPREADING AGENTS; EACH FIGURE IN THE MEAN PER CENT DEAD COLUMN IS THE AVERAGE OF TWO DETERMINATIONS

Spreaders 1/6% plus nicotine sulphate 1:8000	*Aphis rumicis*		*Macrosiphum rosae*	
	Total No. of insects	Mean % dead	Total No. of insects	Mean % dead
Potassium caproate	1083	19.8*	600	27.4*
Potassium caprate	1093	61.8	782	38.7
Potassium laurate	994	70.7	1000	53.0
Potassium palmitate	1042	26.1	745	35.8
Potassium stearate	857	24.8	614	22.7
Potassium oleate	910	90.6	715	79.4
Potassium coconut soap	907	87.7	728	74.0
Potassium fish oil soap	914	65.5	695	78.5
Sulphonated cod oil	948	32.5	721	50.2
Penetrol**	1387	36.0	471	60.3
Check (water alone)	988	1.0	402	1.7

* Differences in the mean dead (in the same species) of 15.6 per cent are probably significant with odds of 30 to 1 that the difference is not due to chance.

** Penetrol is an oxidized petroleum oil.

The question now arises whether it is possible to combine all the toxicity determinations in Table VI irrespective of species. To determine

this question the results (mortality for each test) are statistically analyzed. The complete analysis of variance is shown in Table VII.

TABLE VII

ANALYSIS OF VARIANCE

Variation due to	Degrees of freedom	Sum of squares	Mean square
Replicate	1	74.1003	74.1003
Treatment	10	27632.6242	2763.2624
Aphids	1	2.4112	2.4112
Interaction of treat. × aphids	10	2387.5123	238.7512
Interaction of repl. × aphids	1	84.2911	84.2911
Error	20	1052.4126	52.6206
Total	43	31233.3517	

The variation due to the use of two species of aphids (as expressed by the mean square in Table VII) is less than the variation due to error; but the "mean square" for interaction of treatments and aphids indicates that the two species reacted differently to the treatments. The combination of the results in Table VI irrespective of species, therefore, is questionable.

The limited number of tests in Table VI indicate that for pure soaps combined with nicotine sulphate the oleate is the most toxic to aphids followed by the laurate and caprate which are more toxic than the palmitate, stearate, and caproate. It is of interest to find that the order of toxicity is similar to that obtained with soaps alone. The increase in toxicity obtained with the addition of nicotine sulphate was largely additive; the better spreading soaps, however, gave the greater increase.

During the spring and summer of 1930 many field tests were made with coconut soap and several samples of potash fish oil soaps, with and without the addition of nicotine sulphate. The fish oil soaps were found to be rather variable, some being slightly more toxic than the coconut soap while others were inferior. A summary of the field results shows that coconut soap is slightly more toxic to aphids than the corresponding fish oil soap.

WITH VEGETABLE AND ANIMAL OILS

In connection with soap toxicity it is of interest to note the toxicity of soaps made from some common vegetable and animal oils. The oils, of the best pharmaceutical grade, were saponified with potassium hydroxide. The fatty acids were liberated from the resulting soap by acidifying with dilute hydrochloric acid. The acids, after washing with a small amount of cold water, were made into potassium soaps as previously described. The resulting soaps were tested on several species of insects in a field insectary and in the greenhouse.

It is well known that different samples of vegetable or animal oils vary in composition and therefore also vary in toxicity. In Table VIII, it is found that in every test olive oil soap, the highest in oleate content, is the most toxic. This agrees with the laboratory experiments previously recorded, which showed conclusively that the oleate is the most toxic of the

TABLE VIII

TOXICITY OF VEGETABLE AND ANIMAL OIL SOAPS

Insect and plant	Temp. °C.	Potassium soap made from the following oils	% conc.	Total insects	% dead
A. Tests in field insectary					
A. rumicis L. on dwarf nasturtium	23	Coconut	1.00	680	99.3
		Palm	1.00	668	99.8
		Menhaden	1.00	380	97.6
		Corn	1.00	608	99.5
		Cottonseed	1.00	547	98.7
		Check (water)	—	614	0.8
Hyalopterus arundinis Fab. on plum (*Prunus domestica* L.)	31	Coconut	0.50	665	97.3
		Menhaden	0.50	600	97.0
		Check (water)	—	481	1.5
Hyalopterus arundinis Fab. on plum (*Prunus domestica* L.)	26	Coconut	0.50	703	92.0
		Cottonseed	0.50	364	91.2
		Check (water)	—	655	2.4
B. Tests in greenhouse					
Thrips tabaci Lind. on buckwheat	27	Olive	1.00	77	80.0
		Coconut	1.00	95	67.4
		Menhaden	1.00	72	52.8
		Castor	1.00	102	3.9
		Check (water)	—	127	1.6
A. rumicis L. on buckwheat	27	Olive	1.00	726	93.0
		Coconut	1.00	692	81.2
		Menhaden	1.00	261	74.7
		Castor	1.00	244	34.4
		Check (water)	—	385	1.3
A. rumicis L. on dwarf nasturtium	29	Olive	0.50	1506	92.0
		Coconut	0.50	868	77.1
		Menhaden	0.50	954	78.2
		Castor	0.50	581	19.3
		Check (water)	—	565	0.9
A. rumicis L. on buckwheat	26	Olive	0.25	361	23.5
		Coconut	0.25	399	14.0
		Menhaden	0.25	427	10.5
		Castor	0.25	307	8.4
		Check (water)	—	436	0.9

soaps tested. The castor oil soap, mainly composed of ricinoleic and isoricinoleic acids (6, v. 2, p. 198–208), is found to be decidedly inferior in

toxicity. From these results it would be expected that ricinoleic and isoricinoleic acids are only slightly toxic to insects. There seems to be little difference between coconut, menhaden, and cottonseed oil soaps.

PHYSICAL PROPERTIES OF SOAPS

The possible relation of the toxicity of insecticides to their physical and chemical properties has received much attention. Close correlation of the toxicity with some physical or chemical property would give a laboratory method of measuring the effectiveness of an insecticide and reduce the necessity of conducting many laborious and expensive field tests. O'Kane, Westgate, Glover, and Lowry (8) have discussed surface tension, surface activity, and wetting ability and their effect on the performance of contact insecticides. These authors present evidence to show that in a given series of related toxic compounds which exhibit surface activity, the toxicity is related to adsorption phenomena. Wilcoxon and Hartzell (13) have developed a method of evaluating a spray by means of its spreading coefficient. The spreading coefficient is calculated from measurements of surface tension and angles of contact. Using spray solutions containing nicotine and different spreaders it was shown that the toxicity followed the same order as the spreading coefficient.

In connection with the toxicity results reported for the potassium soaps, surface tension and angle of contact measurements were made. The surface tensions were determined by the drop weight method of Harkins and Brown (3) and the corrections for the size of the drops given in International Critical Tables (6, v. 4, p. 435) were used. The time allowed for the formation of the drops varied between four and five minutes. The glass tip used for forming the drops was the stalagmometer described by Wilcoxon and Hartzell (13). The angle of contact (which is the angle, within the liquid, between the solid surface and the tangent to the liquid surface from the point where solid, air, and liquid meet) of the soap solutions was determined on small blocks of purified paraffin (M. P. 56° to 58° C.) whose surfaces were kept clean by shaving off thin strips with a microtome. This method gives a surface free from irregularities which is readily leveled. The angle formed by a small drop, approximately 1 mm. in diameter, of soap solution when placed on the paraffin block was projected upon a screen and its outline traced. The angles were measured on a number of tracings and the standard error of the mean (2, p. 178–237) calculated by the formula $\sqrt{d^2/n(n-1)}$.

The contact angle given in Table IX is the so-called advancing angle. The transparent soap solutions gave receding angles smaller than the advancing angles. Many of the spreaders gave translucent or opaque colloidal solutions which rendered the determination of receding angles impractical using the method of Wilcoxon and Hartzell (13).

An examination of Table IX shows that it is possible to separate the most toxic from the least toxic soaps by surface tension measurements. The order of toxicity, however, cannot be determined even when working with a homologous series, such as the soaps of the saturated fatty acids, by surface tension measurements. The contact angle of the solutions furnished a better measure of the relative toxicity than the surface tension. Many of the best spreaders such as the fish oil soaps, oleates, and laurates

TABLE IX

SURFACE TENSION AND ANGLE OF CONTACT OF SOAP SOLUTIONS DETERMINED AT 25° C.

Solution	Surface tension in dynes		Angle of contact in degrees	
	Concentration		Concentration	
	1/6%	1/2%	1/6%	1/2%
Potassium caproate	72	71	93 ± 2.2	77 ± 2.1
Potassium caprylate	—	62	—	—
Potassium caprate	45	37	84 ± 5.9	75 ± 2.0
Potassium laurate	19	20	42 ± 4.3	—
Potassium myristate	—	29	—	—
Potassium palmitate	40	36	72 ± 7.6	—
Potassium stearate	57	45	85 ± 2.2	—
Potassium oleate	21	23	33 ± 1.3	40 ± 1.0
Sodium oleate	—	24	32 ± 0.7	36 ± 1.0
Potassium coconut soap	—	23	—	—
Potassium fish oil soap	23	25	—	—
Sulphonated cod oil	36	35	—	—
Penetrol	43	40	—	—
Distilled water	72	—	104 ± 0.9	—

have a minimum surface tension and angle of contact at low concentrations. At these low concentrations their toxicity is low though their surface tension and contact angle indicate good spreading and wetting. For example, at 25° C. sodium oleate at 0.125 per cent concentration has a surface tension of 23 dynes, a contact angle of 31°, and a 23 per cent toxicity to *Aphis rumicis;* while a concentration of 0.5 per cent of the same soap has a toxicity of 83 per cent, with the surface tension increased to 24 dynes and the contact angle to 36°. Neither the contact angle nor the surface tension of the solution is in this case a good index of the probable effectiveness of the contact insecticide.

Wilcoxon and Hartzell (13) and O'Kane, Westgate, Glover, and Lowry (8) have pointed out that increase in depth of penetration of the tracheae is correlated with increased insect toxicity. Immersion of aphids in soap solutions dyed with carbon black usually resulted in some penetration of the larger tracheae. An aphid cleared after immersion in a 0.5 per cent potassium oleate solution is shown in Figure 2. Uncleared aphid specimens examined with a binocular microscope showed similar penetration.

PLANT TOLERANCE OF THE FATTY ACIDS AND THEIR POTASSIUM SOAPS

Plant tolerance is of primary importance since it is as necessary to have a spray which will not injure foliage as it is to have one which will kill insect pests. The fatty acids and their soaps were tested on carefully selected potted plants of uniform size and age. The six species selected

FIGURE 2. Soap (0.5 per cent potassium oleate) darkened with India ink penetrating aphid trachea.

(Fig. 3), indicating all degrees of susceptibility to injury, were tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L. var. Turkish), potato (*Solanum tuberosum* L.), bean (*Phaseolus vulgaris* L.), cabbage (*Brassica oleracea* L. var. *capitata* L.), and nasturtium (*Tropaeolum minus* L.). The plants were grown in the greenhouse and therefore were

FIGURE 3. Fatty acid and soap injury to plants. *Top row:* One-sixth per cent fatty acid injury to nasturtium. A, caproic; B, caprylic; C, capric; D, lauric; E, myristic; F, palmitic; G, stearic; H, oleic (emulsifier, sulphonated cod oil). *Middle row:* Two per cent potassium soap injury to tobacco. I, caproate; J, caprylate; K, caprate; L, laurate; M, myristate. *Bottom row:* One-fourth per cent acid and alkali injury to tobacco. N, caproic; O, capric; P, lauric; Q, sulphuric acid; R, sodium hydroxide (emulsifier, sulphonated cod oil).

probably more easily injured than plants grown outdoors. As in previous experiments, sulphonated cod oil was used as an emulsifier for the fatty acids. It was found necessary to increase the amount of emulsifier when larger amounts of fatty acids were used. A minimum amount of one-fourth per cent of emulsifier was used, but when the fatty acids were used at a greater concentration than one-fourth per cent, equal amounts of fatty acids and emulsifier were used. The methods employed for making the emulsions have been previously described.

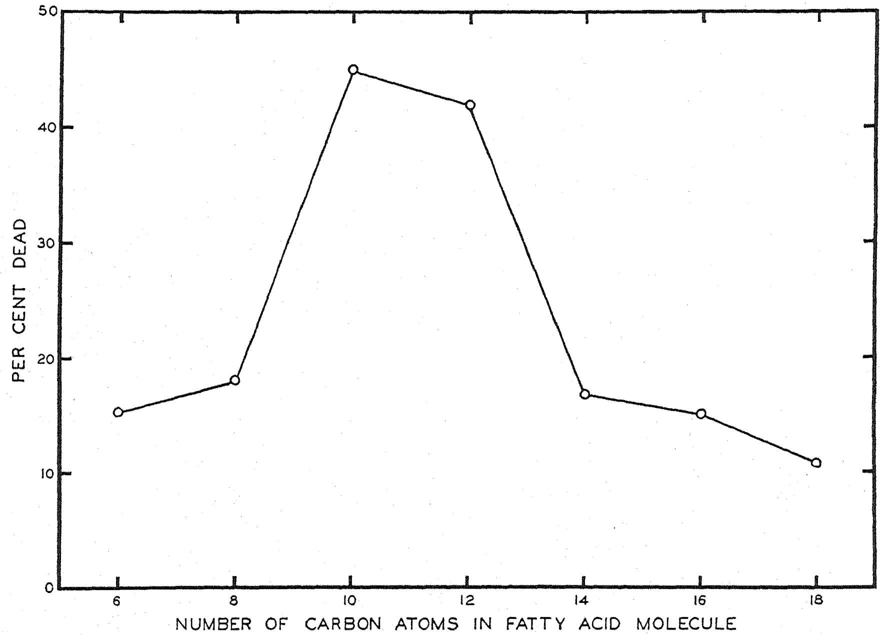

FIGURE 4. Toxicity of one-sixth per cent fatty acids to *Aphis rumicis.*

The plants, after spraying, were placed outside the greenhouse and kept there for a three-week period. Careful readings were taken three times during the first week, and if no injury was apparent at the beginning of the second week the plant was considered non-injured. The potted plants were watered twice daily during the experiment without wetting the leaves. During the period of the tests no rain occurred and the weather was mild with uniform temperature. The results of these experiments with soaps and fatty acids are given in Table X.

Another series of experiments was conducted in the greenhouse where the fatty acids were used at a concentration of one-sixth per cent. In the greenhouse experiments a number of new test plants were used. The following plants were added to those previously mentioned: buckwheat (*Fago-*

TABLE X

TOXICITY OF THE FATTY ACIDS AND THEIR POTASSIUM SOAPS TO VARIOUS PLANTS

A. Toxicity of acids to plants

Spray	Tomato	Tobacco	Potato	Bean	Cabbage	Nasturtium
Caproic ¼%	o*	o	o	++	o	++
Caproic ½%	+++	o	o	+++	o	+++
Caproic 1%	++++	++	+++	++++	o	++++
Capric ⅛%	++++	o	+++	++++	++++	+++++
Capric ¼%	++++++	++++	++++	++++++	+++++	++++++
Capric ½%	++++++	++++++	++++++	++++++	++++++	++++++
Lauric ⅛%	+++	+++	++	+++	o	++++
Lauric ¼%	+++++	++++	++	++++	+++	+++++
Lauric ½%	++++++	+++++	++	++++++	++++	++++++
Palmitic ¼%	o	o	o	o	o	o
Palmitic ½%	o	o	o	o	o	+
Palmitic 1%	o	o	o	o	o	+
Stearic ¼%	o	o	o	o	o	o
Stearic ½%	o	o	o	o	o	o
Stearic 1%	+	o	o	+	o	++
Oleic ¼%	o	o	o	+	o	+
Oleic ½%	++	o	o	++	o	+++
Oleic 1%	+++	o	++	++	o	++++

TABLE X (*Continued*)

Spray	Tomato	Tobacco	Potato	Bean	Cabbage	Nasturtium
H_2SO_4 ⅛%	+	+	o	+	o	++
H_2SO_4 ¼%	+++	++	o	++	o	+++
NaOH ⅛%	o	o	o	o	o	+
NaOH ¼%	+	++	o	++	o	++
Check (water)	o	o	o	o	o	o
B. Toxicity of potassium soaps to plants						
Caproate 1%	+++	+++	++	++	o	o
Caproate 2%	+++++	++++	++++	+++++	o	+++
Caprate 1%	o	+	o	o	o	++++
Caprate 2%	+++	++	+	++++	o	++++++
Laurate 1%	o	o	o	o	o	++
Laurate 2%	o	o	o	++	o	+++
Palmitate 2%	o	o	o	o	o	o
Stearate 2%	o	o	o	o	o	o
Oleate 1%	o	o	o	++	o	++
Oleate 2%	o	o	o	++	o	+++
Check (water)	o	o	o	o	o	o

* o = No injury; + = very slight; ++ = slight; +++ = moderate; ++++ = severe; +++++ = very severe; ++++++ = dead.

pyrum esculentum Moench), castor bean (*Ricinus communis* L.), salvia (*Salvia splendens* Ker-Gawl.), red clover (*Trifolium pratense* L.), and cucumber (*Cucumis sativus* L.). The results obtained agreed with those reported in Table X.

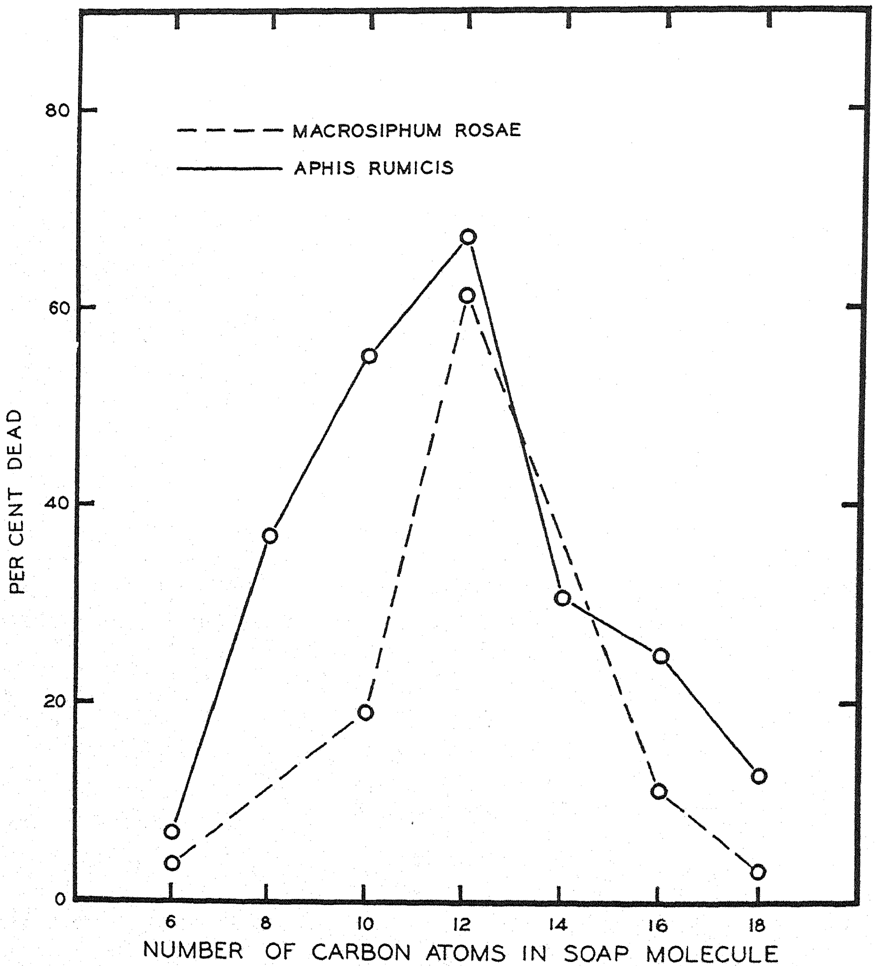

FIGURE 5. Toxicity of 0.5 per cent potassium soaps to *Aphis rumicis* and *Macrosiphum rosae.*

Those fatty acids which are the most toxic to insects (Fig. 4) also cause the greatest plant injury (Table X). With some of the fatty acids, particularly capric and lauric, plant injury may result before any insect control is obtained. Lauric and capric acids were found to be more injurious to plants than an equal percentage of sulphuric acid.

There was found an absence of correlation between plant and insect toxicity with the potassium soaps of the fatty acids. Plant injury decreases as the size of the soap molecule increases. A summary of the plant toxicity of the various soaps is given in Table X and their toxicity to insects indicated in Figure 5.

SUMMARY

The use of soap as a contact insecticide has been discussed.

The toxicity of some of the common fatty acids was determined, using the bean aphid (*Aphis rumicis*) and the rose aphid (*Macrosiphum rosae*) as experimental insects. Capric and lauric acids were found to be more toxic than oleic, caprylic, myristic, caproic, and palmitic acids while stearic was the least toxic of the fatty acids tested.

The insecticidal value of the potassium soaps was determined. The order of toxicity of the soaps was found to be: oleate, laurate, caprate followed by the equally toxic caprylate, myristate, and palmitate which are more toxic than the stearate and caproate. The addition of nicotine to the soap solutions did not alter the order of toxicity. When the soaps and nicotine sulphate were combined it was found that the toxicity due to the nicotine was not strictly additive; the better spreading soaps increased the effectiveness of the nicotine.

The toxicities of the soaps and nicotine sulphate have been compared with some of the commercial spreaders for nicotine.

Potassium soaps made from olive, coconut, castor, corn, palm, cottonseed, and menhaden fish oil were tested on several species of aphids and one species of thrips. Olive oil soap, containing the highest percentage of oleate, was found to be the most toxic.

The surface tension and angle of contact of the potassium soaps were determined. The relation of physical properties to the toxicity of the soaps has been examined.

Tests conducted on six species of plants showed that the order of toxicity of the fatty acids to plants is the same as that obtained with insects. Plant tolerance for the soaps of the fatty acids increased with the increase in size of the soap molecule.

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AGAVE MURPHEYI, A NEW SPECIES

FREDERICK GIBSON[1]

The agave here described, *Agave Murpheyi*, is unlike any other agave of Arizona because the scape starts to elongate in early winter, which differs from the usual beginning of inflorescence in the spring for most of the other species. The scape continues to grow rapidly until cold weather retards the rate of growth and may cause partial injury to the tender tip, but normal growth follows warmer weather and the panicle is completed. Flowers appear in late March and April, some of which may be deformed or turn into bulbous plantlets. After the normal flowers have withered there is another extended period of growth of mixed deformed flowers and bulbils. Very few fertile capsules are produced and the infertile ones are soon crowded off by the bulbils. Reproduction is principally by stolons, whereby dense clumps are formed. Seed dispersal serves for distribution over mountain ranges and desert areas. Chance rooting of fallen bulbils is a third method of propagation.

This species is very rare and apparently becoming extinct, as only four clumps at localities far apart have been reported.

Agave Murpheyi, sp. nov.[2] Acaulescent, widely stoloniferous. Leaves smooth, pale glaucous-green, becoming yellowish-red at time of inflorescence, 6.5 to 10×65 cm., flattish below and widest above the middle, strongly concave near the tip, both faces bearing indentations of adjacent leaves. Spine 4×15 mm., reddish-black fading to gray, grooved only near the base and flattened to the middle, slightly decurrent or to the first or second prickle. Prickles about 50 on each side, same color as the spine, 1 to 3 mm. high on elevations of equal height, curved either upward or downward, usually alternating in size. Inflorescence starts about November 1st. Scape 10 cm. in diameter at base and about 3.5 meters high, ovate paniculate near the top, clothed with spine and prickle-bearing bracts; peduncles about 16, ascending at about 45 degrees, largest 3×21 cm., tripartite; pedicels 3 to 5 mm. high, stout. Flowers very pungently sweet-odored, 6 cm. long, ovary 2.5 cm. long, tube 1.5 cm. long, and 1.3 to 1.7 cm. wide, segments 0.7 to 0.9×1.7 cm.; color pale greenish-yellow slightly

[1] Director, Boyce Thompson Southwestern Arboretum, Superior, Arizona.

[2] **Agave Murpheyi,** sp. nov., acaulis; foliis glabris glaucis basi planis ultra medium latissimis ad apicem valde concavis 65 cm. longis, 6.5–10 cm. latis, spina atrorubra cinereave 4×15 mm. demum basi canaliculata ad medium planata paulo decurrente; aculeis utrinque ca. 50, 1–3 mm. altis rectis vel recurvis; scapo ca. 3.5 m. alto, bracteis spiniferis et aculeatis; paniculae ovatae ramis adscendentibus ramulis robustis bulbillos in bractearum axillis gerentibus; staminibus medio tubo insertis; stylo lobos perianthii aequante demum triplici elongato; capsula basi in stipitem contracta; seminibus nigris 6×9 mm.

tinged with red, especially in bud; inner segments strongly ribbed on the back, all tips incurved. Filaments greenish-yellow, inserted at the middle of the tube, 4.5 cm. long, 2 mm. thick opposite the tips of the segments.

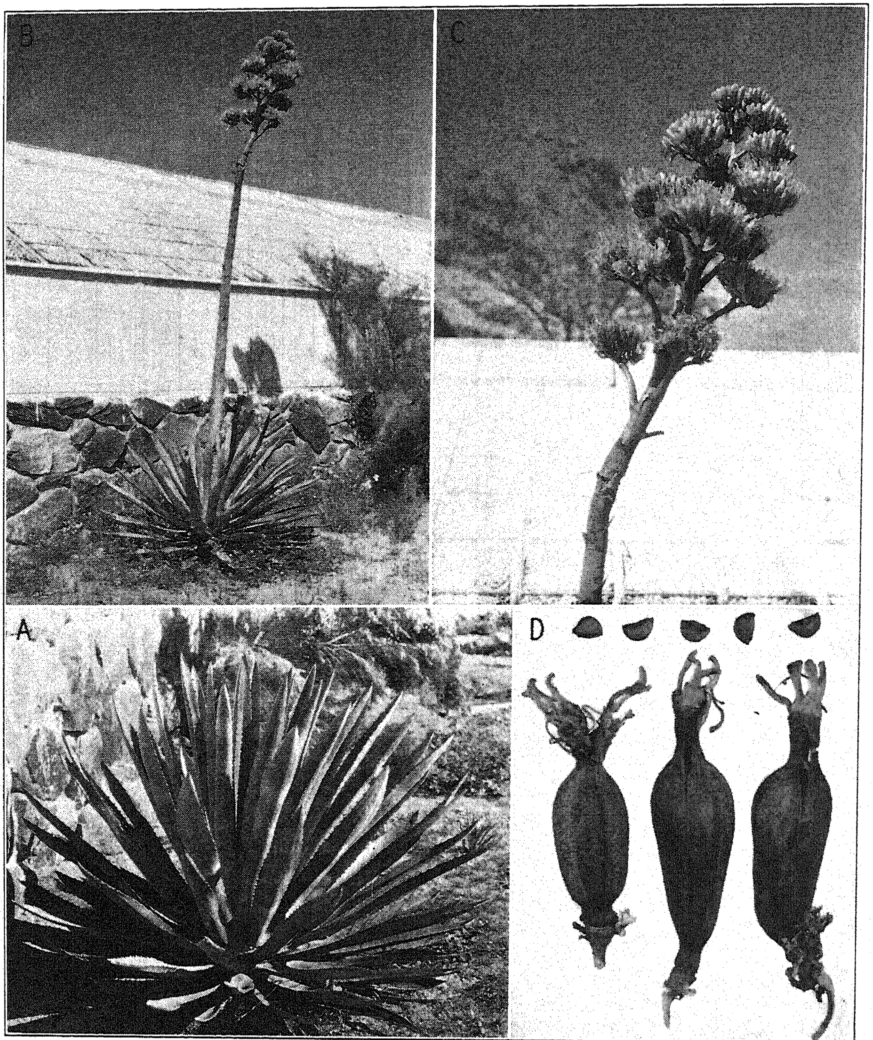

FIGURE 1. A. *Agave Murpheyi*, type specimen in cultivation. Summer, 1933; B. Plant shown in A in flower, spring 1934. The bend in the scape below the first branches of the panicle shows the height attained during the winter of 1933; C. Close view of the panicle; D. Capsules and seeds; secondary growth of deformed flowers and bulbils on the pedicels.

Anthers 3×25 mm., pale brownish-yellow, opening equally from top toward the bottom. Style 0.2×5 cm., equalling the segments at the time of

anthesis and later extending to full length. Capsules 2.5×6.0 cm., attenuate to the stipitate base, dark brownish-black, triangular in cross section, veins prominent, opening about September 1st, perianth persistent. Infertile fruits soon crowded off by bulbils. Seeds dull black, 6×9 mm., thin.

Inflorescence very strongly bulbiferous, lasting into the second or third year. The bulbs form in the axils of small scaly bracts on the pedicels, from adventitious buds, or from the base of other bulbs.

Named for William Calvert Murphey, Superior, Arizona, who is an amateur observer of native plants and animals and who first discovered this species. He noticed that it was unlike most other Arizona agaves because of the bulbiferous nature of the inflorescence and leaf shape.

Habitat. At elevation of 2,000 to 2,500 ft. in central Arizona, on rocky hills. Queen Creek, near Superior (W. C. Murphey); Roosevelt Dam (F. Gibson); Tonto Basin (J. P. Hester); Paradise Valley (J. W. Evans).

Type locality. Near the Boyce Thompson Southwestern Arboretum, Superior, Arizona.

Type specimens. Deposited and cotypes cultivated at the Boyce Thompson Southwestern Arboretum, Superior, Arizona.

Affinities. Near to *Agave palmeri* Engelm. and its orange-flowered relative of central Arizona, but very distinctly different in shape of leaves, color of flowers, and size of seeds.

INDOLE-3-*n*-PROPIONIC ACID AS A GROWTH HORMONE AND THE QUANTITATIVE MEASUREMENT OF PLANT RESPONSE

A. E. Hitchcock

For several years this laboratory has been interested in the formative responses on plants, particularly epinasty and the initiation of roots, as induced by pure chemical substances. The effectiveness of carbon monoxide, ethylene, acetylene, and propylene in causing proliferation, bending, swelling, and rooting on certain plants has already been published (1, 4, 5, 6, 7). If growth substances or hormones are involved in the formative responses to these gases, it is not known whether the special substances are formed as a direct action of the gas on the tissue responding, whether they are formed in one place and are then transported to the region of response, or whether it is a question of the redistribution of the growth substances already present. The fact that more than one pure chemical substance induces these formative responses raises the question as to how many other chemicals are capable of performing the same function.

Through the courtesy of Dr. K. V. Thimann of the California Institute of Technology a few milligrams of a synthetic preparation of the hetero-auxine growth substance (β-indolyl-acetic acid)[1] were obtained. Tests with this chemical showed that essentially the same formative responses were produced on tomato as were caused by the four gases previously mentioned. It is assumed that this is the same growth substance originally isolated and synthesized by Kögl and his associates (2). Due to the limited amount of this chemical at hand for experimental work, it was decided to procure a closely related homologue and attempt to convert it to the acetic acid form. Consequently, 2 grams of indole-3-*n*-propionic acid (β-indolyl-propionic acid) were purchased from the Eastman Kodak Company as their No. 2530 with a stated M.P. of 132° to 133° C. According to a determination in our laboratory the M.P. was found to be 133.4° C., and that given by Richter (3, p. 65) is 134° C.—indicating that the Eastman product is a reasonably pure preparation of indole-3-*n*-propionic acid.

Although Kögl and his associates state definitely that in terms of the "Avena-Einheit" units the propionic form is inactive (2, p. 94 ". . . es ist sehr bemerkenswert, dass auch die homologe β-indolyl-priopionsäure keinerlei Wirksamkeit zeigt"), it was compared in this laboratory with the synthetic preparation of β-indolyl-acetic acid and was found to be of

[1] The writer has been informed recently that Dr. Richard H. Manske, National Research Council, Ottawa, Canada, stands ready to synthesize either indolyl-acetic acid or indolyl-propionic acid with a few weeks' notice.

approximately the same effectiveness in causing bending, swelling, proliferation, and rooting, particularly on potted plants of tomato and African marigold. Although the activity of indole-3-*n*-propionic acid has not been determined in terms of "Avena-Einheit" units in this laboratory, its ability to cause certain formative responses described in the present paper (not to be confused with coleoptile bending) indicates a high activity from this standpoint. Regardless of any traces of impurities which the Eastman product may contain, its activity in producing these formative effects, and the fact that it may be purchased in amounts at least of from one to ten grams at a quoted price of $1.20 per gram, should be of interest to those who are desirous of working with this type of chemical.

Preliminary tests with both growth substances (β-indolyl-acetic acid and β-indolyl-propionic acid) showed that certain bending responses, particularly downward bending (epinasty) of the leaves of tomato and tobacco, might be used as a means of measuring the relative effectiveness of different growth-promoting chemicals or of preparations containing them. The application of the chemical to the plant requires only a few seconds and the response occurs in a few hours in light or in darkness and at ordinary greenhouse temperature (21° to 26° C.), so that the test is an extremely simple procedure. Since these experiments were carried out during the month of December at a time when the poorest growth conditions are encountered, it seems apparent that seasonal differences should not interfere with the tests described.

METHODS

The growth substance was taken up in lanolin (U. S. P.) and was also dissolved in distilled water Tap water preparations were not tried. Lanolin preparations were applied in a thin film by rubbing the mixture with a glass rod on the epidermis of the region to be treated. In certain tests the water preparations were introduced by means of a small glass tube having a capacity of approximately 0.4 cc. and drawn to a capillary at one end. The capillary end of the tube was so inserted in the tissue as to be self-supporting and at the same time allow a slow flow of the liquid into the tissue over a period of several hours. Another method consisted in placing the basal ends of tomato cuttings in the water preparations containing indole-3-*n*-proprionic acid. For these particular tests the stems of four- to eight-inch tomato plants, growing in flats, were cut off just above the cotyledons and were then placed in the test solutions. Glass vials two inches tall and seven-eighths of an inch in diameter or flasks of 125 cc. capacity were used to hold the solutions. Ten cubic centimeters of the test solution were placed in the vial and 25 cc. in the flasks. Any leaves on the lower two inches of the stem were removed. There were at least two leaves four to five inches long remaining on the upper portion of the stem

of each cutting. Marigold cuttings were tested in a similar manner, except that the basal cut was made farther up the stem through succulent tissue.

Indole-3-*n*-propionic acid is only slightly soluble in distilled water. One hour of continuous shaking was necessary to dissolve completely 10 milligrams in 500 cc. of water. When shaken continuously for two days, 424 milligrams dissolved in one liter of distilled water at 25° C. The highest concentration of this particular growth substance in water preparations used in these experiments was 20 milligrams per liter. From this stock solution three dilutions were made so that each was one-fifth the concentration of the preceding one. Other concentrations were also used. The dilution series most frequently used contained the following amounts of indole-3-*n*-propionic acid per liter: 10, 2, 0.4, and 0.08 milligrams. These same amounts of this chemical were also used per gram of lanolin. The maximum amount of the growth substance per gram of lanolin was 50 milligrams. It was found that a lanolin preparation containing two milligrams per gram was noticeably active without causing extreme types of bending, swelling, and proliferation, and it was this concentration which induced formation of roots on stems of the marigold.

The species of plants used in these experiments were: tomato (*Lycopersicon esculentum* Mill.), African marigold (*Tagetes erecta* L. vars. Lemon Queen and Orange), tobacco (*Nicotiana tabacum* L. var. Turkish), buckwheat (*Fagopyrum esculentum* Moench.), and dahlia seedlings (*Dahlia variabilis* Desf.).

EXPERIMENTAL RESULTS

RESPONSE TO LANOLIN PREPARATIONS

Leaves. When the preparation was applied on any one side of a tomato petiole over a distance of one-fourth to one-half inch, the direction of bending was away from that side. An example of downward bending (epinasty) is shown in Figure 1 A. The degree of bending, the rate of bending, and the speed of recovery depended mainly upon the concentration of growth substance. The amount of preparation applied and the area and length of the region covered were also limiting factors, but to a lesser degree. With high concentrations bending commenced in two and one-half hours and progressed for at least 24 hours. Little or no recovery occurred over a period of several days. In the case of low concentrations bending commenced in three to five hours and recovery was complete or nearly so in 24 to 48 hours. The exact time for maximum bending was not determined. Young leaves near the top of the plant usually showed more curling than leaves lower down. The approximate minimum concentration causing epinasty was 0.08 milligram per gram of lanolin. Preparations containing 2, 0.4, and 0.08 milligrams could be readily distinguished by the degree of epinasty on young tomato plants two to four inches in height

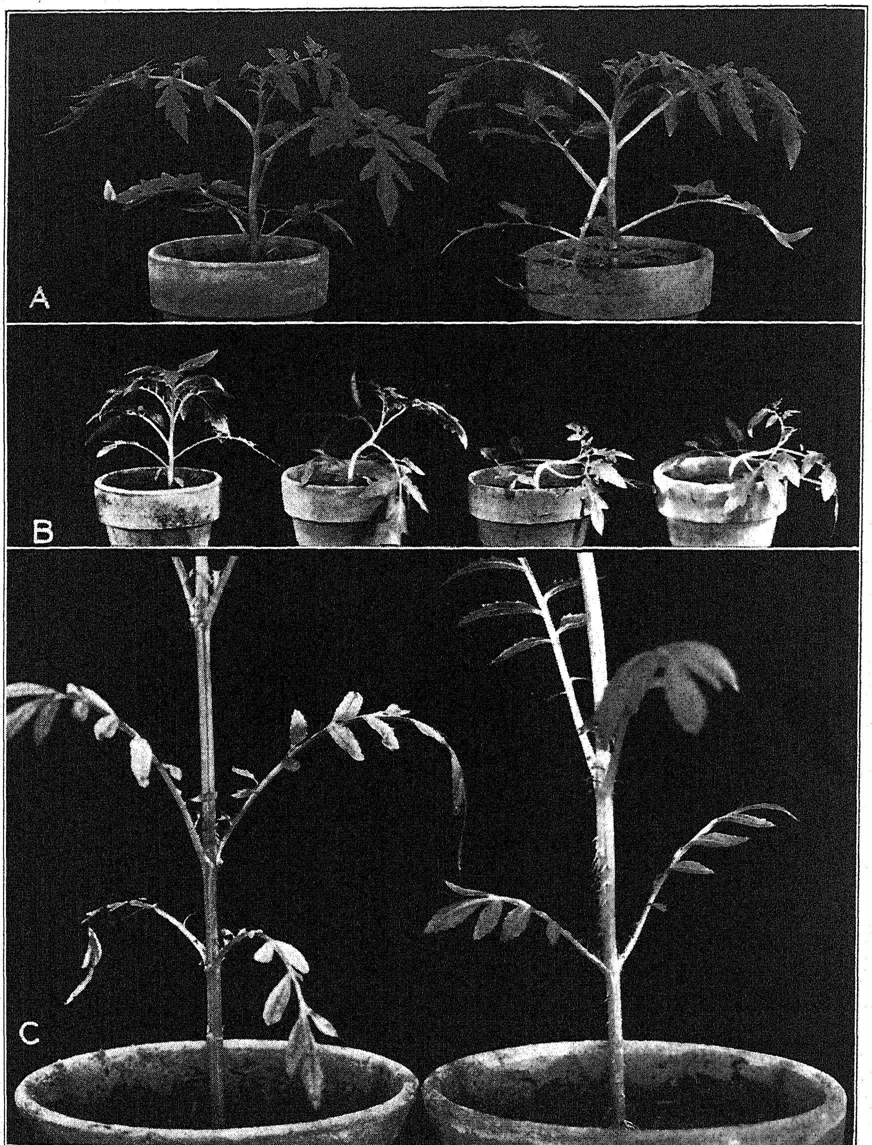

FIGURE 1. Types of bending and rooting induced by application of lanolin containing either hetero-auxine or indole-3-*n*-propionic acid (control plant on left in each case). A. Downward bending of middle petiole of right-hand tomato plant after chemical was applied to upper side at point of bend. B. Stem bending caused by application of chemical to one side of tomato stem. C. Roots on marigold from region treated with chemical (left side of internode above lower pair of leaves). Roots emerged later on all sides of stem below.

or on marigold plants four to eight inches tall. With these concentrations the epinastic response to each of the three dilutions was approximately the same for both growth substances. The application of lanolin not containing the growth substance caused neither bending nor any visible change in the plant.

High concentrations of growth substance applied near or at the base of the petiole caused the stem to bend in the region of, or at a point below the treated petiole, and occasionally there was some bending of the lower leaves. After at least some recovery had occurred, the preparation on the petiole was still active as evidenced by a bending response when the mixture was transferred from the treated petiole to other plants not previously treated. Noticeable swelling of the portion of the petiole treated, occurred in 16 hours but became more pronounced after several days. More swelling occurred on the side treated than on the opposite side. With the higher concentrations of growth substance whitish patches, which sometimes covered the entire treated region, developed in 16 to 48 hours. As in the case of swelling, the whitening of the tissue became more pronounced in the course of several days. Raised areas resembling advanced root primordia usually developed on the treated region. Roots appeared after 10 to 14 days from the treated region near the base of the petiole on some of the tomato and marigold plants. On one of these marigold plants and on several tomato plants, roots also appeared from the second internode below the treated leaf.

Stems. Application of the growth substance to one side of the stem caused bending away from the treated side (Fig. 1 B). When applied to any portion of the stem of a four-inch tomato plant, the growth substance caused bending. Stems of tobacco, marigold, dahlia, and buckwheat were treated only on the succulent portions from one to several inches above the soil, but marked bending occurred in all cases. Treatment of the entire surface of one internode on tomato stems (eight to ten inches tall) with a lanolin preparation containing ten milligrams of indole-3-*n*-propionic acid per gram caused the stem to bend and also resulted in a pronounced epinastic response on all leaves. When marigold was treated in a similar manner, all leaves did not respond. The tobacco showed no epinasty on leaves above or below the treated portion of the stem. Recovery of the stems to an upright position usually occurred in a few days with the lower concentrations of growth substance. The highest concentration used (50 milligrams of indole-3-*n*-propionic acid per gram lanolin) produced a permanent bend and caused the stem of tomato to split open on the treated side after a period of seven days. Adjoining tissue was whitish and later became brown. The diameter of the stem at this point was approximately twice that of the control stem.

β-indolyl-acetic acid induced roots to form on the stems of tomato,

marigold, and buckwheat from only the treated region, after 10 to 14 days. This response is shown for marigold in Figure 1 C. In the case of marigold, roots appeared later, on all sides of the two internodes below the one treated. Buckwheat and tobacco showed the most localized response in rooting, since roots appeared only from the region treated which was on one side of the stems 12 inches above the soil. These particular plants were not placed in a high relative humidity until after root-like projections had developed.

The shortest time for roots to appear on tomato and tobacco stems resulting from treatment with lanolin containing indole-3-*n*-propionic acid was eight days. In this case roots emerged near the treated region or on the opposite side. These plants received the highest concentration of growth substance (50 milligrams per gram of lanolin), and were the ones on which the stem split open. Two days later roots had emerged from the treated region which by this time showed marked injury.

When marigold plants were placed in a Wardian case immediately after treatment of the stems with a lanolin preparation containing two milligrams of indole-3-*n*-propionic acid, roots first appeared from the treated internodes on the sixth day. In this series of tests the lanolin preparation was applied entirely around the stem. Different plants were treated either on the first, second, third, or on the first and second internodes above the cotyledons. Plants on which only the third internode was treated, showed the most pronounced rooting response, followed in decreasing order by the plants treated on the second and first internodes, respectively. The African marigold is particularly well suited for root initiation tests because control plants six to eight inches high can be subjected to a high relative humidity for at least two weeks without the appearance of roots on any portion of the stem. Optimum conditions for root initiation have not as yet been determined. The minimum concentration for rooting was likewise not determined.

RESPONSE TO WATER PREPARATIONS

Leaves. When the solution was introduced by means of glass tubes, bending of the leaves was downward (epinasty). The amount of solution introduced in the petiole ranged from less than 0.1 cc. to 0.4 cc. On this basis the amount of growth substance causing epinasty in the case of the most frequently used stock solution was 0.001 to 0.004 mg. and in the case of the lowest (1/25) active dilution was 0.00004 to 0.00016 mg. No attempt was made to cause other types of bending by restricting the point of entry of solutions to a particular region. The response of individual leaves to water preparations of the growth substance applied at the base of the petiole was essentially the same as that caused by the lanolin preparations. Although swelling occurred, it was less pronounced than that

resulting from the lanolin mixture. Quantitative measurements of the epinastic response to the growth substance showed that the degree of declination and the rate of recovery of tomato and tobacco leaves were significantly different for three concentrations in a series in which the amount of growth substance was one-fifth of that in the preceding dilution. More than one tobacco leaf on the same plant could be used to determine the epinastic response to low concentrations of the growth substance. Results of these tests will be published in a separate paper.

High concentrations of growth substance (2 to 20 mg. per liter) caused leaves lower down the stem to respond, and in young tomato plants the stem also bent. In a few tests the glass tube was inserted on the midrib two and one-half inches from the base of the petiole. Arched bending occurred mainly near the base of the petiole, a distance of approximately two inches from the point where the solution entered. When responses occurred any appreciable distance away from the point of entry of the solution, they were mainly below the point of entry toward the stem.

Stems. The few tests in which the solution was introduced in the stem internodes of tomato, caused mainly bending of the stems and little or no bending of the leaves. Roots appeared on stems of marigold when the tube containing the growth substance passed through the base of the petiole into one side of the node. Although most roots appeared below this point, a few came out one-half inch above. Control plants treated similarly with distilled water showed no rooting on any portion of the stem.

In certain tests the stems of tomato plants, grown in flats, were cut off just above the cotyledons and were then placed in distilled water containing amounts of indole-3-*n*-propionic acid ranging from 10 milligrams to 0.08 milligram per liter. Definite epinasty had occurred at the end of one and one-half hours on leaves of cuttings placed in the highest concentration. After a total of six hours a pronounced epinastic response had occurred on these cuttings. A slight response was evident at this time on cuttings in the solution containing 2 mg. Bending was mainly of the arched type, and was not localized at or near the base of the petiole during the first eight hours. After this time, any further bending was usually localized near the base of the petiole. The maximum distance from the cut surface to the base of the uppermost leaf showing marked epinasty was eight inches. The height of the solution column was one inch or less. Cuttings of marigold responded in a similar manner to the same concentrations, except that the response was much slower. No pronounced epinasty was evident in six hours, but at the end of 16 hours (overnight) the leaves on the second node above the cotyledons showed marked epinasty. Roots appeared in five days from the basal portion of the tomato and marigold cuttings in distilled water and in the solution containing 0.08 mg. indole-3-*n*-propionic acid per liter. The higher concentrations retarded rooting.

RESULTS WITH OTHER CARRIERS

Some recently tested preparations in which olive oil, paraffin oil, maize oil, castor oil, and Amalie oil (a commercial mineral oil) were used as carriers for indole-3-*n*-propionic acid were found to be more effective than lanolin preparations containing the same amount of the chemical. With an olive oil preparation a measurable bending response occurred on tomato within one hour.

SUMMARY AND CONCLUSIONS

Indole-3-*n*-propionic acid obtained from the Eastman Kodak Company induced the same bending, swelling, proliferation, and rooting responses on certain plants as resulted from similar treatment with a synthetic preparation of hetero-auxine. Three methods are described for determining the relative effectiveness of different growth substances in causing cell elongation resulting in the downward bending (epinasty) of leaves: (a) rubbing a lanolin preparation of the growth substance on the surface of petioles or stems by means of a glass rod, (b) introduction of a water preparation by means of small glass tubes inserted in the petiole tissue, and (c) placing the basal ends of tomato cuttings in a water preparation of the growth substance. These tests represent an extremely simple procedure, the application of the growth preparation requiring only a few seconds, and the time for initial response from 2 to 16 hours. No special equipment or technique was required, and the bending responses occurred in light or in darkness in the greenhouse where the temperature was 21° to 26° C.

Quantitative measurements of the epinastic response on tomato and tobacco leaves showed that significant differences in the degree of bending and the rate of recovery occurred when the concentrations of growth substance differed by one-fifth. The approximate minimum active lanolin preparation of both growth substances in causing epinasty of tomato leaves was 0.08 mg. per gram of lanolin.

Distinct differences in the localization of the responses were observed according to the concentration of the chemical, the method of application, and the species of plant. Lanolin preparations induced the most localized responses. The most localized rooting response occurred on buckwheat, tobacco, and African marigold. Tests with cut tomato stems in water preparations showed a relatively rapid upward movement of the substance which acted directly or indirectly in producing the epinastic response. The upward movement in tomato stems was much faster than in the marigold. By use of one or more of the methods described it is believed that considerable information may be obtained concerning the direction, rate of movement, and the channels through which growth substances travel.

The fact that one homologue of hetero-auxine was found to be active

in causing certain formative responses, indicates that possibly other indole derivatives and perhaps other unrelated chemicals might induce one or more of these same responses. With this view in mind, other chemicals are being tested, making use of the epinastic and rooting responses, according to the methods described, as a measure of the relative effectiveness of these chemicals.

Note Added in Galley Proof

Before the galley proof of this paper was received, many chemicals had been tested as to their power to induce bending and rooting. Glycollic, pyruvic, and malonic acids consistently induced bending of tomato petioles. Benzoic, citric, oxalic, and tartaric acids also caused bending but they were less effective than the first three named. All of these chemicals were effective only at or slightly below the concentration causing injury. Two other chemicals, phenylacrylic acid ($C_6H_5 \cdot CH:CH \cdot COOH$) and phenylpropionic acid ($C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH$) induced bending, swelling, proliferation, and rooting responses similar to those caused by indole-3-*n*-propionic acid. Effective concentrations of phenylpropionic acid ranged from 8 to 40 mg. per gram of lanolin. Roots appeared on stems of potted tomato and African marigold plants six to seven days after treatment. Phenylacrylic acid was much less effective since higher concentrations (100 to 200 mg.) and a longer period for rooting (8 to 12 days or longer) were required. The approximate minimum concentrations of the indole and phenyl derivatives which induced rooting on stems of tomato were as follows: synthetic hetero-auxine 0.4 mg., indole-3-*n*-propionic acid 2 mg., phenylpropionic acid 8 mg., and phenylacrylic acid 100 mg. per gram of lanolin. Bending responses caused by the two phenyl derivatives were about the same but much less pronounced than those caused by the two indole derivatives. Thus there are at present four groups of substances of a distinctly different chemical structure which induce the same types of bending and rooting on the tomato and African marigold: the indole derivatives β-indolyl-acetic acid and indole-3-*n*-propionic acid, the two phenyl derivatives mentioned above, carbon monoxide, and the unsaturated hydrocarbon gases (ethylene, acetylene, and propylene).

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PRODUCTION OF ETHYLENE BY PLANT TISSUE AS INDICATED BY THE EPINASTIC RESPONSE OF LEAVES[1,2]

F. E. DENNY AND LAWRENCE P. MILLER

Evidence is accumulating in favor of the view that ethylene is produced in the normal life processes of plants and is given off from the tissue into the surrounding air. The starting point for this phase of plant physiology may be found in the observation of Elmer (5) that when apple fruits are stored in the same container with potato tubers a delay in the sprouting and an abnormal development of the sprouts ensues, caused by an emanation from the apples. Subsequently, Huelin (8) showed that effects similar to that given by apples could be obtained by using ethylene, and he suggested that ethylene was the effective emanation from apples. Botjes (1) enclosed an apple with a tomato plant in a bell jar and obtained the epinasty (downward curvature of petioles) of the leaves found by Harvey (7) and Doubt (4) to be produced when certain leafy plants are exposed to air containing extremely low concentrations of ethylene. Finally, Gane (6) completed the proof by isolating ethylene from apple tissue by appropriate chemical methods, and furnishing conclusive evidence of its identity in the vapor surrounding the fruit. He also refers to leaf-epinasty experiments, the results of which were in press but which have not yet come to the attention of the writer.

This is a preliminary paper giving the results of some experiments with various plant tissues, using the epinastic response of leaves as an indication of the presence of ethylene in the emanations from the tissue. Positive responses were obtained with fruits of apple (*Pyrus malus* L.), pear (*Pyrus communis* L.), tomato (*Lycopersicon esculentum* Mill.), banana (*Musa paradisiaca* var. *sapientum* Kuntze), cantaloupe (*Cucumis melo* L.), squash (*Cucurbita moschata* Duchesne.), eggplant (*Solanum melongena* L.), avocado (*Persea americana* Mill.), and loquat (*Eriobotrya japonica* Lindl.); with seeds of lima bean (*Phaseolus limensis* Macf.) and pea (*Pisum sativum* L.), using the partly mature seeds from the fresh unripe pods; with flowers of dandelion (*Taraxacum officinale* Weber); with the crown and leaves (without roots or flowers) of dandelion; with leaves of dandelion, rhubarb (*Rheum rhaponticum* L.), yellow calla (*Zantedeschia elliottiana* Engler), and hollyhock (*Althaea rosea* Cav.); with the leafy stems of peony (*Paeonia albiflora* Pallas); and with the young shoots or stems of asparagus (*Asparagus officinalis* L.).

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 92.

[2] This article was preprinted May 31, 1935.

The most suitable plant found for use as a test plant for showing the the epinastic response was the young potato (*Solanum tuberosum* L.) plant about four to eight inches high. Tomato plants were also used but were found to be much less sensitive than potato plants. The potted potato plant and the plant tissue to be tested for the effect of its emanations were placed in a container which was then sealed for 16 to 20 hours, usually in darkness.

The response called epinasty of leaves is shown in Figure 1. Figures 1A and 1B illustrate the result of exposing potato plants to the emanations from varying amounts of squash tissue and dandelion flowers. Under these same conditions a similar response is obtained with about 2 g. of apple tissue.

The epinasty caused by the other tissues was similar to that shown in Figure 1, and was indistinguishable, at least in appearance, from that caused by ethylene itself.

In these preliminary tests it is only with the apple, squash, and dandelion tissues (which appeared to be unusually effective) that a study was made of the relation of the response to the quantity of tissue taken. With the other tissues the amount taken varied from 25 to 200 grams, and the volume of air space within the container was usually 7 liters.

The tomatoes, squash, dandelion, rhubarb, calla, peony, and hollyhock were obtained from the greenhouses or gardens of the Institute; the asparagus shoots were harvested from fields in Maryland and were transported promptly by automobile to the laboratory in sealed tin containers; the bananas were furnished by the Research Department of the United Fruit Company;[3] the avocado, cantaloupe, eggplant, and loquat fruits, and peas in pods were shipped from California by refrigerated express in sealed tin containers, each kind in a separate container;[4] the apples, pears, and lima beans were obtained from public markets, since at the time of the experiments they were not available freshly-harvested.

The results with the fruits and vegetables obtained from public markets are less dependable because the previous history of the tissue was not known and there was no assurance that it had not been in contact either with ethylene, or with some other tissue from which it could absorb the emanation. However, the tests were repeated several times on different days and with tissues obtained from different markets. In all cases an epinastic response to these tissues was obtained.

Some tests were made of the possibility of contamination of one tissue

[3] Thanks are expressed to G. L. Poland of the United Fruit Company for furnishing this dependable supply of green bananas.

[4] For these supplies we are indebted to E. M. Chace and W. B. Davis of the Laboratory of Fruit and Vegetable Chemistry of the United States Department of Agriculture, Los Angeles, California.

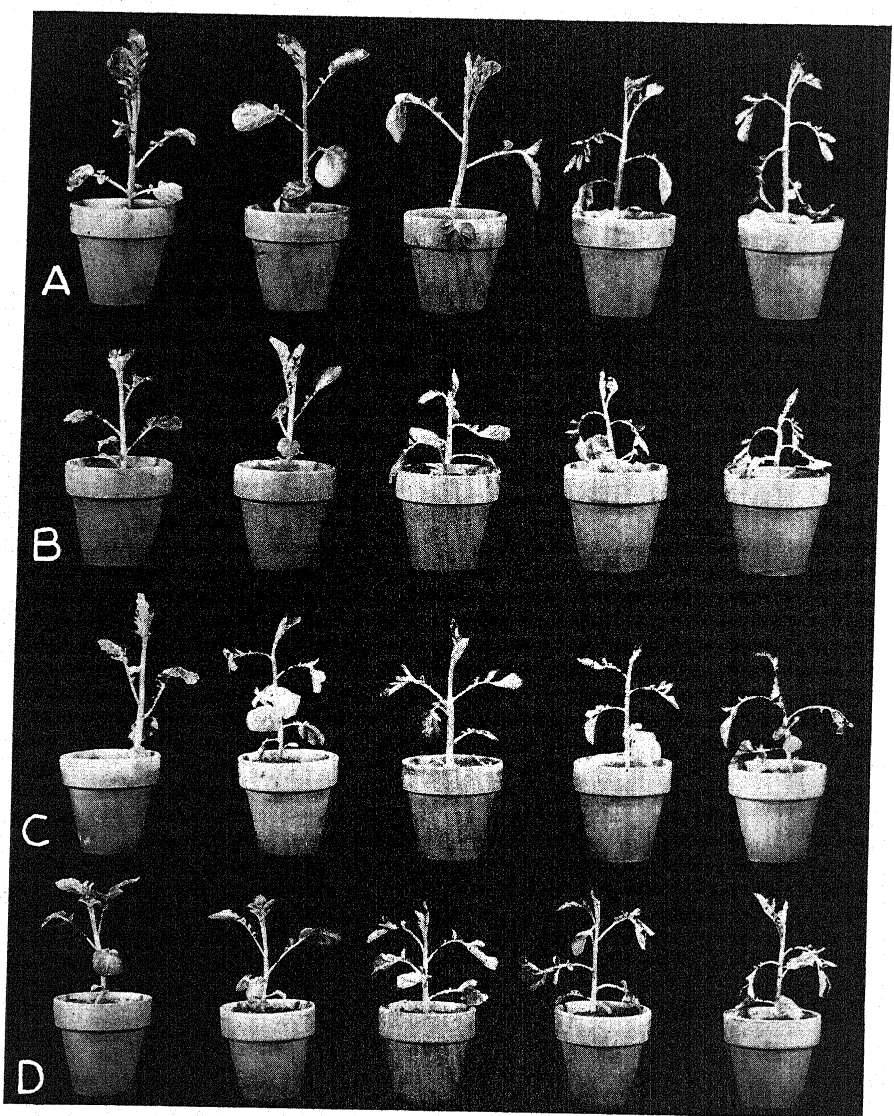

FIGURE 1. Epinasty of potato leaves induced by emanations from plant tissue. A. Effect of dandelion flower heads (av. wt. 0.6 g. each); left to right: check, 3 dandelion flowers, 6 flowers, 12 flowers, 24 flowers. B. Effect of squash fruit tissue; left to right: check, 2 g. of tissue, 6 g., 18 g., 54 g. C. Effect of leaves and tops; left to right: check, peony tops, hollyhock leaves, rhubarb leaves, dandelion leaves. D. Effect of tip and base tissue of edible young shoots (6 in.) of asparagus; left to right: check, tip tissue without KOH solution for absorbing CO_2, tip tissue with KOH, base tissue without KOH, base tissue with KOH.

by emanation from another, and it was found that a tissue which did not give the epinastic response could acquire the capacity by being in contact with a tissue that could induce it. Thus, a turnip (*Brassica rapa* L.) root which at first failed to cause epinasty of potato leaves, readily did so if it was stored overnight in a closed container with apples, bananas, or tomatoes. And this capacity was not lost by one day's exposure to air previous to a second test on the same turnip root.

In the experiments with the leaves of rhubarb, calla, and hollyhock, with the stems of peony, and with the crown and leaves of dandelion, usually 50 to 200 g. (fresh weight) of tissue were placed in containers of 10 l. capacity. The petioles or cut stems were placed in beakers of water, a layer of KOH solution (2 N) for absorbing CO_2 was placed at the bottoms of the containers, and provision was made for a continuous supply of oxygen to replace that removed by respiration, using O_2 bottles connected to a constant-level water supply. This arrangement permitted the emanations from the tissue to accumulate, and kept the plant in good physiological condition for responding to them. Figure 1 C shows the response obtained with rhubarb, dandelion, and hollyhock leaves, and with tops of peony.

The asparagus stems (50 g.) were cut into one-half inch pieces and were placed in 7 l. jars with the potato test plant. Better responses were obtained if a layer of KOH solution was used to absorb the excess of CO_2 produced in respiration. Bases of asparagus shoots gave better tests than tips (see Fig. 1 D).

Elmer (5), Gane (6), Smith and Gane (10), and Kidd and West (9) dealt with emanations from fruit, and even emphasized the importance of the ripening stage of the fruit for these effects. And in the present tests these responses with tomatoes, bananas, and loquats were obtained not with immature fruits but with fruits starting to show at least slight coloration. But in view of the positive tests obtained with leaves, stems, and flowers it is believed that the production of the emanation is not restricted to fruit tissue. In fact, even with fruits we must consider the possibility that the immature as well as ripe fruits produce the emanation, although in lower amounts, and that the differences are quantitative rather than qualitative.

A number of the plant tissues tested including fruits, stems, leaves, roots, and flowers did not cause epinasty of leaves. These species will not be enumerated at this time, since their failure to induce the reaction may be due not to their failure to produce the emanation, but to the production of it in concentrations too low for a positive test under these conditions. Attempts are being made to arrange experimental procedures which will cause an increase in the metabolic rate and permit an accumulation of the emanations.

Admittedly these epinastic responses do not furnish complete proof that ethylene is produced by the tissue, or that it is the sole factor causing these effects upon the test plants. Final proof must wait upon the completion of procedures for the isolation of ethylene from the atmosphere surrounding the tissue and its identification by approved chemical methods.

In the meantime, however, there are strong reasons for believing that the effective constituent is ethylene. In the first place, Gane (6) has identified ethylene as an emanation from apple tissue, and the response induced by these other tissues are identical in appearance to that produced by apple tissue and by pure ethylene. Secondly, Crocker, Zimmerman, and Hitchcock (3) in an investigation on the effect of various chemicals found only five gases which produced epinasty of tomato petioles: ethylene, propylene, acetylene, butylene, and carbon monoxide. Of these ethylene was by far the most effective, propylene and acetylene requiring 500 times the minimum concentration for response with ethylene, carbon monoxide requiring 5000 times this concentration, and butylene 500,000 times. Thus, ethylene is the only one which is effective in very low concentration.

Now, in these tests the apple tissue appeared to be more effective than any of the other tissues tested, squash fruits and dandelion flowers being the only ones that approached apple tissue in efficiency. But the measurements of Gane (6) indicated that the ethylene production was "perhaps of the order of 1 cubic centimetre during the whole life history of the fruit." If we interpret this as referring to a single apple (approximately 200 g.) for a period of four weeks during which the emanations of the apple were absorbed in Gane's experiments, and if (as found in the present tests) 2 g. of apple tissue in a 7 l. space will produce within one day a quantity of ethylene sufficient to cause epinasty, we find that on this basis the amount of ethylene within the 7 l. container should be about 1 part (by volume) in 20 million. In a paper which is to follow in this journal Crocker (2) shows that the potato plant will give an epinastic response in an ethylene concentration of this order, or even in a somewhat lower concentration. The responses in the present tests, therefore, are consistent with the provisional estimates made by Gane (6) for the production of ethylene by apples. And, in view of the evidence that the other tissues in these tests were either considerably or at least somewhat less effective than apple tissue, the indications are that the emanation is present only in low concentration, and that the effective constituent is ethylene.

There is no intention, however, to exclude the possibility that the results may be due to the formation not of ethylene but of other substances. The proof that some product of metabolism other than ethylene is the effective constituent would not detract but rather add to our interest in the problem. But any evidence that some other chemical is the principal factor must be accompanied by proof that ethylene was not present as an

impurity in amounts sufficient to give a concentration of at least 1 part of ethylene in 20 million of air.

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SPECTROGRAPHIC DETERMINATION OF CALCIUM IN PLANT ASHES

KATHARINE WAY[1] AND JOHN M. ARTHUR[2]

Good spectrographic determinations of impurities in metals have been reported by a number of workers, but little use has been made of emission spectrum methods for the quantitative analysis of biological materials. Hilger (5) has issued bibliographies covering both fields. Since the method should prove valuable in the determination of the constituents of plant ashes where chemical analyses, especially for elements present in small amounts, are often tedious and unreliable, it was decided to make further study of the possibilities of its application. Calcium was selected as the first element for study since chemical determinations for comparison could be made easily and accurately.

EXCITATION OF SAMPLE

The spectra of the constituents of a metallic sample can be secured easily by making the sample into electrodes and passing an arc or spark between them. Biological ash, however, cannot be excited in any simple way and must be mixed with a conductor or be introduced as a solution or a powder into an exciting source.

Although good results have been reported by Lundegårdh (8) from spraying solution into a flame this method has not been tried since even an oxyacetylene flame does not reveal any of the non-metals and gives a very much poorer spectrum than the arc or the spark of the metals which it does excite.

Spark methods for solutions were used extensively by earlier workers. Baly (1, v. 2, p. 119–123) has given a good summary of this work. Endeavors to duplicate these methods resulted in excessive spattering and breakage due to the force of the spark. However, Gerlach and Schweitzer (4) have described means for avoiding these difficulties.

The introduction of a solution or a powder into the arc can be accomplished very conveniently by drying out a small amount of the solution on one of the electrodes or by pressing the powder into a hole drilled into it. The powder method although favorably reported by Tourtellotte and Rask (12) was discarded since it was found to result in scattering and since it was felt that different powders could not be packed into holes in precisely the same way. Lewis (7) has described a method for making a

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[2] The writers are indebted to W. D. Stewart for the chemical analyses and to W. J. Youden for assistance in the construction of the microphotometer.

powder into a pellet with a pellet-making machine and burning these uniform pellets in the arc. This method necessitates the weighing of small quantities of material of the order of 10 milligrams and is therefore much less convenient than the method in which solutions are measured and dried on electrodes. This last method, used by Nitchie and Standen (9) and others, is the one which has been applied to plant ashes in the present investigation.

Copper, silver, carbon, and graphite of considerable purity are available as electrodes. Salts dried out on the metallic electrodes were found to be scattered by the arc. Graphite was selected for the work in preference to carbon since the graphite arc wanders about more freely. A good quality of graphite can be secured at reasonable cost so that each electrode can be discarded after one burning.

It was found, however, that the exact way in which a salt solution was allowed to dry on the graphite electrodes made considerable difference in the absolute and relative blackening of the spectral lines. This has not been pointed out previously. If the solution does not wet the electrode it can dry out in either a glassy or chalky condition. If it wets the electrode it can be dried in such a way that a few crystals are visible or that no salt at all is visible. This last condition was selected as the one which could probably be most exactly reproduced. Acidified solutions wet the electrodes much more readily than pure water solutions. After considerable experimentation it was found that complete disappearance of the salt could be consistently achieved if the solutions contained nitric acid in the amount of 1 part in 15, by volume. One-twentieth cc. of solution of approximately the correct concentration, as described later, is dropped on the top of the electrodes and the electrodes dried out in a 55° C. oven. The solution is added to the electrode by means of a capillary pipette and the practice is to put on only a very small drop at first, wait until this has wet the surface of the electrode, and then add the remainder. After the electrodes have dried for one hour in a 55° C. oven, they are placed for one-half hour in a 115° C. oven to insure the volatilization of the free acid. The electrodes, about three-quarters of an inch long, are broken from the long rods supplied by the manufacturer so that the ends are as flat as possible, and the solution added to the resulting rough surface. The sharp edge is filed down a little to keep the arc from playing there too much. If the entire end is smoothed down with a file the solution is likely to leave a ring. Various sizes and shapes of holes for the solution have been tried but they have not been as successful as the rough flat surface just described. Occasionally, electrode rods are found which when treated in the above way show salt crystals on the top or wetting down the side. Such rods are discarded. The method could undoubtedly be improved if electrodes of uniform physical constitution could be found.

The impregnated electrode of 6 mm. diameter is made the lower and positive electrode. The upper negative electrode is 10 mm. in diameter. Having the upper electrode wider than the lower helps to prevent the arc from creeping up the side. An inexpensive type of arc stand is used with specially made holders for the different sized graphites. This is shown in Figure 1 A.

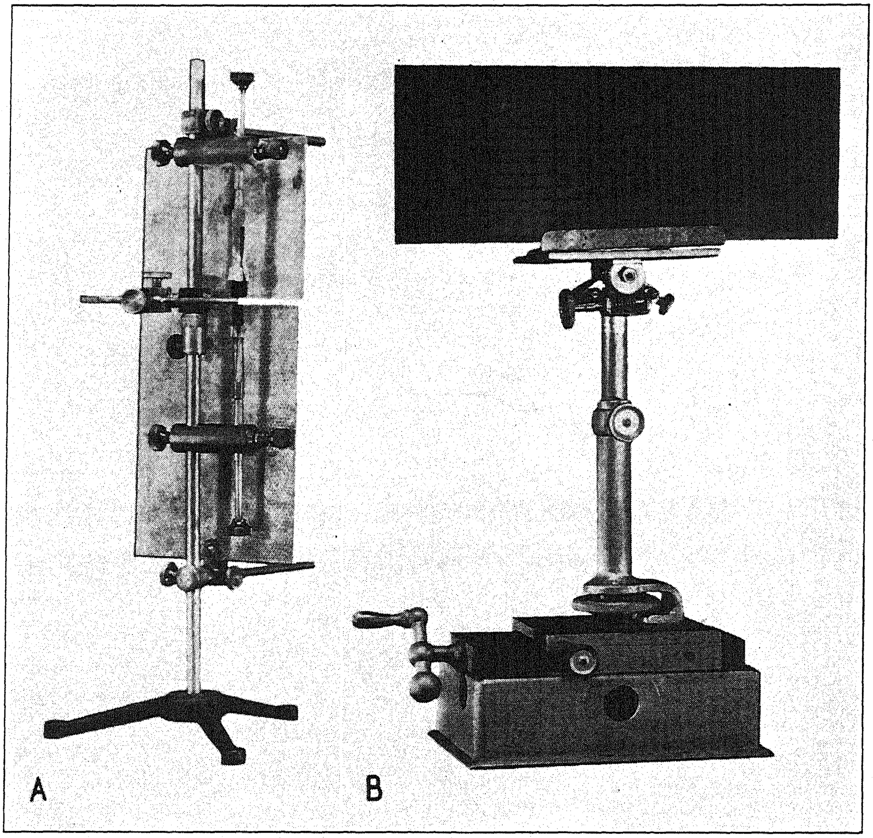

FIGURE 1. A. Photograph of electrode holder showing transite shields for eliminating light from electrodes and pointer arrangement for centering arc. B. Plate holder for microphotometer showing mechanical adjustments used in focusing specific lines on the slit of the photoelectric cell box.

The arc is operated from 125-volt D.C. mains. The current is fixed at six amperes at the beginning of the burning of the impregnated electrodes. It gradually falls to about four and one-half amperes when the salt has been burned off. The electrodes are burned until the lines being used for the determination show no further blackening. The necessary time is found

by burning impregnated electrodes for different times, striking the arc again immediately after each burning and exposing a fresh portion of the plate, and then selecting the burning time for which the repeat burning shows no blackening of the lines in question. The adequacy of the selected burning time is constantly checked by repeat burnings. With our standard ash the exposure is 1 minute, 15 seconds. No attempt is made to keep the current or the voltage across the arc constant.

PHOTOGRAPHY

A number of workers use condensing quartz lenses between the arc and the spectrograph slit. Since the arc wanders it is necessary when a condenser is used to have an arrangement for constantly moving either the arc stand or the lens in order to keep the image of the arc directly on the slit of the spectrograph. This work has been done without a condenser and to minimize the shifting of the arc it is placed at a distance of 55 inches from the slit. This, of course, requires larger amounts of sample than is necessary with the lens arrangement. The lens also protects the slit from the light emission of the hot electrodes which would otherwise give rise to a continuous background in the spectrogram. To eliminate this, shields of transite are placed just in front of the electrodes. A pointer device which can be swung about on the arc stand makes it very simple to find the correct positions for fresh electrodes once the system has been carefully lined up. The distance between the shields is 3 mm. and that between the electrodes 4 mm. The electrode stand, transite shields, and pointed device are shown in Figure 1 A.

In order to secure lines wide enough so that their blackenings can be conveniently measured with the microphotometer described below, it is necessary to use a fairly wide slit. The lines must also be of uniform blackening over their entire width. This can be achieved by proper relation of slit width to collimator lens stop. Stockbarger and Burns (10) and van Cittert (2) give very helpful discussions of this relation. For the distance of the arc and the instrument used, Hilger E 3 quartz spectrograph, lines of good width and uniformity at wave lengths 5330 Å and 5350 Å are secured by opening the slit until four secondary maxima are visible on each side of the principal maximum and then closing down a lens stop specially installed for the purpose between the collimator lens and the prism until only half of the first secondary maxima can be seen.

Cramer Spectrum Process Plates are found to give particularly good results for the two lines mentioned above which are the ones used in the calcium determination. These plates are not very sensitive in the region of these lines and give a minimum amount of continuous background here. The lines themselves can be made sufficiently strong by using the proper amount of solution on the electrodes. More sensitive plates, of course,

require less solution but were found to show frequent background smudges which could not be eliminated. In the ultra-violet where there is little continuous background under any conditions this difficulty would not arise.

"INTERNAL STANDARD" METHOD

The method for determining calcium from the spectrograms is that of an "internal standard" as described by Twyman (13, p. 39) and used by a number of workers. In this method the blackening of a line of the element to be determined is compared with the blackening of a line of an element present in fixed amount in all samples. The lines of a comparison pair should lie close together on the plate in order to minimize effects associated with change of wave length and plate variation. Assuming the plates used similiar to those studied by Lay and Cornog (6), the error in the difference in blackening of two lines one millimeter apart due to inequalities in the plates can be as high as 1.5 per cent which may give rise to an even greater error in the amount of the element being determined. The proper current should be found above which the difference in blackening of the comparison pair is fairly constant and a current slightly greater than this should be used. Moreover, the difference in blackening of the pair should not be affected by small changes in the other constituents of the samples. The absolute blackening of a line is affected by changes in current and changes in the other constituents of a sample. Since these errors are eliminated if a good comparison pair can be found, the internal standard method is more accurate than the method of comparing the blackening of a line in the spectrum of the unknown with the blackening of the same line in the spectra of standard solutions. Since no element is present in plant ashes in fixed amount it was necessary to choose an element not already present in the ashes or present only in very minute traces which could be added to every sample to furnish a suitable line for comparison with a calcium line. The pair of lines at wave length 5330 Å of strontium and 5350 Å of calcium were found to make a very reliable pair. Another pair at wave lengths 2399 Å Ca and 2428 Å Sr proved much less reliable probably because these are both principal series lines and therefore readily absorbed.

A series of standard solutions containing between 0.266 per cent and 0.533 per cent Ca and 1.75 per cent Sr and the other major constituents of the ashes to be analyzed in approximately proportionate amounts is made. The error in the ratio of the amount of Ca to Sr in these solutions as prepared with pipettes is estimated as ± 0.5 per cent. The spectra of several of these standard solutions are taken on each plate with the spectra of a number of unknown ash solutions. The differences in blackening for the lines of the comparison pair in the standard solutions are determined with

the microphotometer described below and plotted against the logarithms of the calcium concentration. This gives very nearly a straight line. The differences in blackening of the comparison pair in the unknowns are then found and the calcium concentrations of the unknown solutions determined from the curve for the standards. If several plates give very nearly the same differences in blackening for the standard solutions, these values are averaged and a mean standard curve drawn for determining all the unknowns on these plates. The temperature and concentration of the developer are carefully controlled to make different plates comparable, but from time to time markedly different values for the standard solutions are obtained due probably to differences in the porosity of the electrodes used or possibly to differences or changes in the plates.

It is necessary, of course, to arrange the dilution of the unknown ash solutions so that the calcium concentration is in the above range. The proper dilution can usually be determined for a number of ashes by one or two preliminary tests.

The elements in the standard solutions are all in the form of nitrates with the exception of phosphorus which is added as H_3PO_4. In order to make the unknown ash solutions comparable the ashes are dissolved in nitric acid, the sulphate precipitated with $BaCl_2$, the chloride removed by repeated drying out with concentrated nitric acid, the strontium added, the solution then made up to the required volume, and the degree of acidity found necessary for wetting the electrodes. If potassium is present in large amounts its lines will interfere with the comparison pair and at least partial precipitation is necessary.

The blackening of a line is defined as the difference in the microphotometer galvanometer throws for clear plate and line; the density as the logarithm of the opacity. As pointed out by Duffendack *et al* (3), the relation between blackening or density and incident light intensity should be determined for the plates used. The step diaphragm method for obtaining this relation suggested by them was tried, but a step diaphragm could not be cut accurately enough to give good results. In this work blackenings rather than densities were used and the straight-line range of the blackening-log. intensity curves for the plates was determined by burning first one, then two, three, etc., electrodes for each spectrum and observing for which numbers burned the difference in blackening of the comparison pair was constant. It was found that when three electrodes were used for each spectrum the blackenings fell in the straight-line range of the plate. Since it is felt that the burning of three electrodes for each spectrum gives a spectrum which is equivalent to the mean of three spectra and thus saves room on the plate and time in evaluating the spectrogram no attempt has been made to alter the current or the concentration so that a smaller number will give the requisite blackening.

MICROPHOTOMETER

The principle of the microphotometer described here is the same as that used by Nitchie and Standen (9) and Lundegårdh (8) in constructing their instruments. Gerlach and Schweitzer (4) describe a slightly different scheme.

A diagram showing the arrangement of apparatus is shown in Figure 2. A strong beam of light is focused on the photographic plate by means of a Bausch and Lomb Research Microscope Lamp containing a 6-volt, 108-watt ribbon filament lamp and condensing lens. To assure steady illumination the lamp is run on three 6-volt storage batteries connected in parallel and charged while in use. The illuminated portion of the photographic plate is focused on the covering of the box containing the photoelectric cell

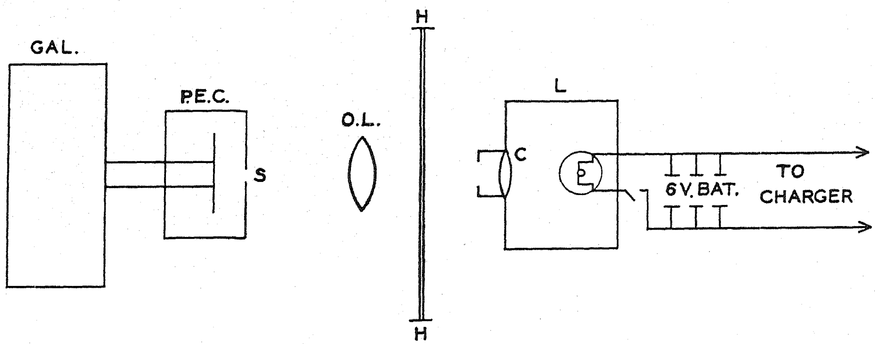

FIGURE 2. Microphotometer arrangement for comparing photographic blackenings—horizontal cross section. Gal.—glavanometer with lamp and scale enclosed; P.E.C.—photoelectric cell in box with adjustable slit S; O.L.—objective lens; H—holder for photographic plate (shown in Fig. 1 B): L—lamp box with condensing lens C.

by a Carl Zeiss microplanar lens, $f=5$ cm.; F/4.5. The slit in the box covering admits light to a Weston photronic cell which is connected directly to a Leeds & Northrup table galvanometer with enclosed lamp and scale. The galvanometer sensitivity is 50 mm. per microampere. The spectral lines are made parallel to the vertical slit in the cell box covering by adjustment of the plate holder shown in the photograph (Fig. 1B) and images of the lines in question are brought over the slit, which is made smaller than the line image, by horizontal movement of the holder. When the line image covers the slit, the galvanometer reading is recorded.

The zero of the galvanometer used has been changed from the center to the extreme left of the scale, so that the whole scale can be used. A large magnifying glass is clamped in front of the scale to make the reading easier. The practice is to focus on the slit a perfectly clear portion of the plate just above or below the lines in question and adjust a diaphragm in front of the condensing lens until the galvanometer reading is 40 mm. The plate

holder is then moved up or down until the lines are parallel to the slit and the galvanometer readings for the blackenings due to the lines and their immediate continuous backgrounds are taken. No correction is made for the background.

RESULTS

Table I shows four galvanometer readings of the same pair of lines with estimations of the percentage of Ca in the ash solution made from these readings while Table II gives four Ca determinations of one ash solution made from four pairs of lines on four different plates.

TABLE I

CALCIUM DETERMINATIONS FROM DIFFERENT READINGS OF SAME PAIR OF LINES

Gal. readings		Difference	% Ca	% Ca (Chem. analysis)
λ5330	λ5350			
22.1	19.4	2.7	0.621	
21.9	19.2	2.7	0.621	0.610
22.0	19.5	2.5	0.608	
21.9	19.3	2.6	0.614	
			Mean = 0.616 ± 0.005	

A comparison of spectrographic and chemical determinations of calcium in several ash samples is given in Table III. The spectrographic determinations given here were made in each case from a single pair of lines. Table II shows the closer agreement with chemical results obtained by averaging values from several pairs. The chemical method used was that of Tisdall and Kramer (11).

TABLE II

CALCIUM DETERMINATIONS FROM FOUR DIFFERENT PLATES ON THE SAME SOLUTION OF PLANT ASH

Gal. difference	% Ca	% Ca (Chem. analysis)
−2.3	0.387	
−1.9	0.403	0.394
−2.0	0.400	
−2.7	0.367	
	Mean = 0.389 ± 0.006	

The errors to be expected in the spectrographic method are those due to (a) inaccuracies in the standard solutions, (b) inequalities in the photographic plates, (c) errors in microphotometer readings, (d) error due to

change of the constants of the photographic plates with wave length, (e) differences in burning conditions, and (f) differences in the distribution of the salt in the electrodes due to differences in porosity. (a) is estimated as giving rise to an error in the final result of ±0.7 per cent, (b) of ±2 per cent, (c) of ±1 per cent. These errors would give an average deviation of about ±2 per cent. The error due to (d) has not been determined for the plates used. The error due to (e) is minimized by proper choice of current as suggested by Duffendack (3). Its magnitude has not been estimated in the present case. Inaccuracies due to (f) are probably the greatest but these could be reduced if more uniform electrodes could be found.

TABLE III

COMPARISON OF SPECTROGRAPHIC AND CHEMICAL DETERMINATIONS OF PERCENTAGE OF CALCIUM IN DRY WEIGHT OF PLANT ASHES

Material No.		% Ca, spectro-graphic	% Ca, chemical	Mean for samples 1 and 2, spectro-graphic	Mean for samples 1 and 2, chemical	Difference between spec. and chem. means
35	Sample 1	2.37	2.53	2.46	2.58	−2.6%
	Sample 2	2.55	2.63			
36	Sample 1	2.35	2.38	2.40	2.34	+2.6%
	Sample 2	2.44	2.30			
43	Sample 1	2.48	2.58	2.47	2.57	−2.6%
	Sample 2	2.46	2.55			
44	Sample 1	2.67	2.65	2.58	2.62	−1.5%
	Sample 2	2.49	2.58			

CONCLUSION

The results show that the spectrographic method described here can be used to determine the amount of calcium in a plant ash within at least 5 per cent of chemical methods. It seems possible, therefore, using the general technique described to work out reliable methods for the determination of aluminum, manganese, iron, and other elements whose chemical determinations in plant ashes present considerable difficulties. Success will depend, of course, on the finding of good comparison pairs.

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CARBON DIOXIDE STORAGE. VIII. CHEMICAL CHANGES IN POTATO TUBERS RESULTING FROM EXPOSURE TO CARBON DIOXIDE[1]

NORWOOD C. THORNTON

The effect of carbon dioxide upon living tissue is a subject offering much interesting information. The stimulating effect of carbon dioxide upon human respiration and the necessity of its presence to maintain the alkaline reserve of the blood has been demonstrated by Henderson (5, 6). It has been observed (7, 8) that the gas has much the same effect upon some plant tissue in that it stimulates respiration and brings about a more alkaline condition within the tissue. Treatment of fungous hyphae with carbon dioxide causes an increase in pH of the cytoplasm (10). Concurrent with that investigation flowers were exposed to carbon dioxide in the presence of oxygen and here again the pH was increased as evidenced by the change of color of the anthocyanin present in the tissue (11). It is evident from these results that the reaction takes place within the living protoplasm. The treatment of non-living tissue with carbon dioxide results in an acidifying effect of the carbonic acid; therefore, it is readily realized that carbon dioxide exerts some control over living processes.

The information reported in this paper is the beginning of a study of the effect of carbon dioxide on the chemical processes going on in living tissue in an attempt to understand by what means the various changes already observed are brought about.

MATERIAL AND METHODS

Non-dormant potato tubers (*Solanum tuberosum* L.), variety Green Mountain, harvested in Maine in the fall of 1934 were used during January to March 1935 in this investigation of the effect of carbon dioxide in plant tissue held at a temperature of 21° C. The carbon dioxide, oxygen, and nitrogen used to make up the gas mixtures were obtained from cylinders of the compressed gases. The storage containers and procedures for carrying out the experiments on respiration were the same as used in previous work (7). In the tests in which the storage period was longer than four days the gases in the cans were completely removed and a fresh gas mixture of the original composition was introduced. This precaution was taken at intervals of four days, and is absolutely necessary in order to insure a high percentage of oxygen within the container at all times. If the oxygen supply is depleted during the storage period considerably different results will be obtained from those reported in this paper.

[1] Presented before the Division of Biological Chemistry at the 89th meeting of the American Chemical Society, New York, New York, April, 1935.

At the end of the storage period the tubers were scrubbed free of all foreign matter and wiped dry with cheesecloth. Tissue to be used for investigation of leaching of electrolytes from the tissue was obtained according to the method used by Guthrie (4) which is as follows: Cylinders of tissue were cut from the tubers with a 14 mm. cork borer and then cut again crosswise with the same cork borer. Ten pieces of tissue obtained in this manner were washed three times with distilled water, held in distilled water for five minutes, and washed again. The excess of water was then wiped from the tissue with dry cheesecloth. The potato tissue was then placed in large test tubes with 25 cc. of distilled water, closed with rubber stoppers, and rotated for four hours at room temperature (24° C.). This period of leaching was found to give the most consistent results. At the end of the leaching period the solution was poured into a Freas cell for the conductivity measurements. In all cases a duplicate tube was set up for the purpose of obtaining leachings for boiling before determining the conductivity in order to eliminate the dissolved carbon dioxide. The boiled leachings were brought to the same temperature and volume as the unboiled leachings before the conductivity measurements were made.

The remaining portions of the tubers were ground through a food grinder, and the juice squeezed out through several layers of cheesecloth. The pH determinations were then made upon this juice. After centrifuging aliquots of the juice were added to boiling water, then made up to a known volume upon cooling. The solutions taken were then cleared with lead acetate and deleaded with sodium oxalate and the reducing sugars were determined before and after inversion with HCl in the cold (1, p. 273). For the sugar determination the Munson and Walker method was used (1, p. 380–381), and the cuprous oxide was titrated with a potassium permanganate solution which had been standardized with a sugar solution of known concentration.

For the catalase determination a 2 cc. aliquot of centrifuged potato juice was taken and diluted to 50 cc. with distilled water and 10 cc. aliquots of this dilution were taken for the determination. The apparatus and procedure described by Davis (2) were used except that the dioxygen was neutralized by $CaCO_3$ instead of NaOH.

Determinations of the reduction of methylene blue, reduction of iodine in acid solution, and tests with sodium nitroprusside were carried out on the centrifuged juice according to the methods used by Denny, Miller, and Guthrie (3).

RESULTS

The treatment of the potato tubers with carbon dioxide in the presence of oxygen for various periods of storage resulted in very pronounced changes in the rate of respiration and chemical composition of the tissue. The data in Table I show a marked increase in the rate of respiration of the potato

TABLE I

EFFECT OF CARBON DIOXIDE, IN THE PRESENCE OF 20 PER CENT OF OXYGEN, ON THE RATE OF RESPIRATION OF POTATO TUBERS HELD AT 21° C.

Days of CO_2 treatment	Hours of respiration test	Mg. O_2 uptake/kg./hr.		
		Control	30% CO_2	60% CO_2
5	25	8.8	10.3	31.4
5	18	6.3	6.5	29.5
8	20	7.6	7.8	40.6
8	20	5.7	9.7	32.8

tubers when held in 60 per cent of carbon dioxide for periods as long as five to eight days. The maximum increase in the rate of respiration during these tests was 475 per cent and minimum 256 per cent over that of the control. It was found that storage periods of more than eight days did not greatly alter these results and shorter storage periods gave correspondingly smaller increases in the rate of respiration. The treatment with 30 per cent of carbon dioxide gave a small but consistent increase in the rate of respiration of the tubers. As one would expect the respiration rate had been gradually increasing since the day after the potatoes were first exposed to the carbon dioxide. These respiration tests were made during the last few hours of the storage period; therefore, the data represent the maximum rate of respiration for only a short period of the total treatment.

Concurrent with the increase in respiration of the potato tissue there was observed a decided increase in the catalase activity of the pressed and centrifuged juice obtained from the tubers. The data in Table II show the catalase activity of the juice expressed in cc. of oxygen produced in five

TABLE II

EFFECT OF CARBON DIOXIDE IN THE PRESENCE OF 20 PER CENT OF OXYGEN ON THE pH AND CATALASE ACTIVITY OF POTATO TUBERS

Days of CO_2 treatment	pH of juice upon extraction			Catalase activity of extracted juice; cc. of O_2 in 5 min.		
	Control	30% CO_2	60% CO_2	Control	30% CO_2	60% CO_2
1	5.90	5.87	5.90	12.4	16.0	17.0
3	5.85	5.93	6.17	11.2	11.7	13.5
4	6.00	6.15	6.31	12.7	12.5	13.6
7	6.09	6.07	6.27	9.1	13.5	16.3
11	6.09	6.17	6.41	6.3	12.6	13.7
12	6.04	6.17	6.37	7.9	7.2	16.2
18	6.04	6.17	6.64	11.8	11.5	19.3
21	6.10	6.24	6.63	10.3	13.7	20.3

minutes. With 21 days of treatment there was found an increase over the control amounting to as much as 97 per cent. In the case of shorter periods of treatment the increase in catalase was found to vary considerably, averaging approximately 50 per cent although in one four-day test the

increase was only 7 per cent. Catalase activity was increased with the treatment with 30 per cent of carbon dioxide, but not to the extent as with the higher percentage of carbon dioxide.

Correlated with the increase in respiration and catalase of the potato tubers there was found a very decided increase in the pH of the potato tissue. The information obtained from tests made upon the pressed juice is shown by the data in Table II. The results show that the carbon dioxide has acted indirectly upon the potato tissue through activation of respiration and other changes to bring about a very marked chemical change in the tissue. The increase of as much as 0.6 of a pH unit of the potato juice is observed only if oxygen has been present during the test period, and only when dealing with a living organism. If oxygen was absent, or if the carbon dioxide came into contact with the potato juice after its removal from the tuber, an acid condition resulted which has been discussed in previous work (8). Whether this pH change is responsible for the observed differences in catalase is not definitely known, but further work is being carried out upon this phase of the problem.

TABLE III

EFFECT OF CARBON DIOXIDE IN THE PRESENCE OF 20 PER CENT OF OXYGEN ON THE SUGAR CONTENT OF POTATO TISSUE

Days of CO_2 treatment	Mg. in 2 cc. of centrifuged juice					
	Reducing sugars			Sucrose		
	Control	30% CO_2	60% CO_2	Control	30% CO_2	60% CO_2
3	27.1	28.2	40.0	2.9	8.5	7.5
4	24.7	21.6	29.8	3.9	6.4	7.0
5	16.6	13.9	18.8	7.2	9.9	14.4
6	24.1	32.2	36.3	3.4	12.6	16.0
7	33.8	30.2	33.8	3.9	9.5	16.0
11	22.2	25.6	44.0	4.4	7.7	19.1
12	27.1	30.2	33.3	5.5	10.5	22.7

Further changes within the potato tuber are evident by the very marked increase in the sugar content as shown by the data in Table III. The outstanding change is to be found in the sucrose content of the treated tubers over that of the control tubers. This increase varied from approximately 100 per cent with the short period of treatment to approximately 350 per cent for the longer periods of storage. Furthermore, it is to be observed that even with 30 per cent of carbon dioxide there resulted a definite increase in sucrose content of the potato tubers. The increase in reducing sugars, calculated as dextrose of this variety of potato treated with various concentrations of carbon dioxide, is not consistent but varied with different periods of storage.

The results of a further investigation of the effect of carbon dioxide

on potato tissue is shown in Table IV. These data indicate that the treatment may alter the permeability of the potato tissue to a considerable extent if the period of exposure is for more than six days. The longer the period of the gas treatment the greater was the increase in conductivity of the leachings of the potato tissue. In order to eliminate the dissolved carbon dioxide interfering with the true result the leachings of a duplicate sample were boiled before the conductivity determinations were made. In nearly every case the conductivity increased rather than decreased as would have been expected if the observed changes were due merely to the presence of carbon dioxide in the leachings. Although hydrolysis of some substances leached from the tissue may be the contributing factor for this change upon boiling, the identity of the constituents responsible for this change has not yet been determined.

TABLE IV

EFFECT OF CARBON DIOXIDE IN THE PRESENCE OF 20 PER CENT OF OXYGEN ON THE LEACHING OF ELECTROLYTES FROM POTATO TISSUE

Days of CO_2 treatment	Specific conductivity of leachings $\times 10^{-5}$					
	Unboiled			Boiled*		
	Control	30% CO_2	60% CO_2	Control	30% CO_2	60% CO_2
1	51.9	51.5	52.8	57.3	51.2	55.0
1	48.7	48.7	49.2	58.8	50.4	55.8
2	51.7	45.8	53.3	60.8	55.4	62.8
3	53.8	50.5	50.7	56.9	57.0	59.4
6	49.8	47.5	79.7	44.5	49.7	76.5
11	50.9	77.5	93.8	42.7	89.4	118.8
12	51.2	67.4	112.1	56.8	90.7	135.8
18	51.2	108.5	129.0	57.2	105.8	120.0
21	53.4	106.9	180.0	60.5	104.5	235.7

* A duplicate was boiled to expel carbon dioxide, then cooled to temperature of the unboiled sample before determining specific conductivity.

The carbon dioxide treatment of the potato tubers brings about a considerable change in the reducing properties of the potato juice (9). In tests with freshly-harvested potatoes the rapidity of reduction of methylene blue and the development of a positive test with sodium nitroprusside were quite evident as the concentration of carbon dioxide was increased. The amount of iodine absorbed by the juice from the treated potatoes was double the amount absorbed by the controls. With older, non-dormant potatoes the increase in activity of the reducing properties of the potatoes is not so marked with the carbon dioxide treatments. In these tests only slight differences were obtained with the methylene blue reduction and iodine absorption tests while no definite test was obtainable with the sodium nitroprusside.

SUMMARY

Potato tubers, variety Green Mountain, were treated with various concentrations of carbon dioxide in the presence of 20 per cent of oxygen for various periods up to 21 days at 21° C. Increases in the rate of respiration and specific conductivity of leachings from the potato tissue were observed. Also, the catalase activity, pH, reducing sugar content, and sucrose in the extracted juice were greatly increased over that found in the control. A slight increase was observed in the reducing properties of the juice.

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RELATIVE GROWTH AND DRY WEIGHT PRODUCTION OF PLANT TISSUE UNDER MAZDA, NEON, SODIUM, AND MERCURY VAPOR LAMPS

JOHN M. ARTHUR AND W. D. STEWART

INTRODUCTION

A more efficient lamp, as regards energy consumption in proportion to the dry weight of plant tissue produced, is desirable as a practical means of forcing plant growth during the winter months. For this reason, it seemed desirable to compare under controlled conditions of temperature, humidity, and light intensity the growth and dry weight produced by a single species of plant when exposed to various light sources now available commercially. An arrangement was made with the General Electric Vapor Lamp Company[1] for furnishing lighting units of neon, sodium, and mercury vapor lamps to give an intensity of the order of 700 to 800 foot candles over an area approximately three feet square.

METHODS OF STUDY

Buckwheat (*Fagopyrum esculentum* Moench.) was chosen for the study because in previous studies (1, 2) it had been observed to grow well under continuous illumination, giving a proportional increase in dry weight with increasing light intensity, within certain limits, and possessing the property of flowering on any day length after a certain period of vegetative growth. The plants were grown from seed in eight-inch pots in the greenhouse until they were three to four inches in height. An average of 16 plants were grown in each pot. The pots of plants were then transferred to the constant light room which has been previously described (2). The air temperature was held at 77° F. within plus or minus two degrees and humidity at approximately 70 per cent relative. Four pots of plants were grown under one type of vapor lamp in each test. At the same time, four check pots of plants were grown under a 500-watt Mazda lamp, fitted with an aluminum reflector. A large sheet of galvanized iron was placed between the two lamps so as to separate the two lighting units and yet give good circulation of conditioned air around each set of plants. The Mazda lamp used in each test was burned at a high filament temperature, that is, a 110-volt lamp, operated on an average voltage of 119, approximating a filament temperature of 3000° K. First the light intensity from the Mazda was brought near an arbitrary reading of 70 millivolts at the soil level by raising or lowering the lamp. This intensity reading was made on a voltmeter connected to a

[1] The writers gratefully acknowledge their indebtedness to R. D. Mailey and L. J. Buttolph of the General Electric Vapor Lamp Company for furnishing lamps and data on current consumption and energy distribution for each lighting unit.

Weston cell through a Wheatstone bridge circuit which allowed a reading of the potential across the Weston cell without using any of the current output of the cell. A 100-ohm resistance coil was also shunted across the terminals of the cell. This reading was obtained when the tip of the Mazda lamp was approximately 31 inches above the soil level. The vapor lamp to be compared was mounted on the opposite side of the metal shield and was then raised or lowered so as to give this same intensity value. No attempt was made to obtain a reading of exactly 70 millivolts for each pair of lamps, but each vapor lamp was compared in every test with the same type of 500-watt Mazda lamp at an intensity closely approximating 70 millivolts, and a set of plants was grown under the Mazda and the dry weights determined whenever a similar test was made with each vapor lamp. On account of the excessive growth in height under the Mazda it was found necessary to raise this lamp approximately two inches at the end of a 4 or 5-day exposure period and again after another 4-day period. The amount raised each time was approximately the same as the difference between the increase in height of the plants under the Mazda and that of the plants under the vapor lamp, and resulted in again equalizing the photo cell readings at the level of the tips of the crown of leaves under each lamp unit.

RESULTS

The plants were cut off at the soil level after a growth period of 8 to 11 days. The total green weight and the green weight of stems and leaves were determined. The tissue was dried in a vacuum oven and the dry weight of stems and leaves determined. The dry weight data for all types of lamps are given in Table I. The green weight data have been omitted for the sake of brevity. They followed closely the variations in the dry weight tables. The dry weight data are assembled first on the weight produced per pot of plants in order to present the variation between pots. This gives a more accurate idea of the value of the average figures. The greenhouse plants were held continuously in an ordinary greenhouse not at all comparable as to temperature with the constant light room. The greenhouse values are included only to show the amount of dry weight which might be expected under ordinary growing conditions of sunlight in the fall and winter months in buckwheat seedlings planted at the same time and grown for the same number of days. The plants in the light room under the various lamps received continuous, 24-hour illumination as compared with less than half this value of sunlight in the ordinary greenhouse. Plants grown under the neon lamp tests showed the greatest variation. This is due to the lack of uniformity of seedlings at the time they were transferred from the greenhouse to the light room. The plants in pot No. 4 under the comparison standard Mazda lamp in the neon tests failed to recover from slow germination in the greenhouse. The indications are

TABLE I

BUCKWHEAT PLANTS; AVERAGE DRY WEIGHTS IN GRAMS PER PLANT PRODUCED UNDER EACH LAMP

Mazda Lamp vs. Sodium Vapor Lamp

	Stems					Leaves					Whole Plant				
	Pot numbers					Pot numbers					Pot numbers				
	1	2	3	4	Average	1	2	3	4	Average	1	2	3	4	Average
Greenhouse	0.038	0.040	0.035	—	0.037	0.065	0.058	0.057	—	0.060	0.100	0.090	1.100	—	0.100
Mazda	0.116	0.102	0.106	0.095	0.104	0.093	0.092	0.093	0.092	0.093	0.210	0.190	0.200	0.190	0.200
Sodium	0.068	0.078	0.081	0.065	0.073	0.100	0.112	0.110	0.095	0.104	0.170	0.190	0.190	0.160	0.180

Mazda Lamp vs. Neon Lamp

	1	2	3	4	Average	1	2	3	4	Average	1	2	3	4	Average
Greenhouse	0.065	0.072	0.082	—	0.073	0.089	0.095	0.087	—	0.090	0.154	0.166	0.170	—	0.163
Mazda	0.193	0.174	0.165	0.126	0.164	0.132	0.140	0.114	0.104	0.122	0.325	0.315	0.280	0.230	0.287
Neon	0.149	0.160	0.144	0.149	0.150	0.156	0.168	0.161	0.176	0.165	0.306	0.327	0.306	0.325	0.316

Mazda Lamp vs. Mercury Vapor Lamp

	1	2	3	4	Average	1	2	3	4	Average	1	2	3	4	Average
Greenhouse	0.022	0.023	—	—	0.023	0.032	0.035	—	—	0.034	0.057	0.058	—	—	0.058
Mazda	0.142	0.124	0.147	0.116	0.132	0.102	0.096	0.109	0.099	0.101	0.244	0.220	0.256	0.215	0.233
Mercury	0.059	0.051	0.049	0.065	0.056	0.099	0.093	0.093	0.107	0.098	0.158	0.144	0.142	0.172	0.154

Ratio of average stems to leaves, Mazda 1.10, Sodium 0.70
Ratio of average stems to leaves, Mazda 1.34, Neon 0.91
Ratio of average stems to leaves, Mazda 1.30, Mercury 0.57

that the neon lamp as used in the test is at least as good as the Mazda lamp in total dry weight production. The dry weights of plant tissue produced were much more uniform in the mercury and sodium vapor tests. It is evident from these tests that the dry weight produced under these two lamps is not as great as under the Mazda. It is evident also that the average dry weight of plant tissue produced under the sodium lamp is only slightly less than that produced under the Mazda whereas the weight of tissue produced under the mercury lamp is considerably less.

The average ratio of stems to leaves is especially interesting. This ratio under the Mazda varied from 1.10 to 1.34. The average ratio of stems to leaves under the neon lamp was 0.91 as compared with 0.70 under the sodium and 0.57 under the mercury lamp. Stated in another way, plants grown under the Mazda lamp had more stems than leaves, under the neon the plants had slightly more leaves than stems, while under the sodium they produced considerably more leaves than stems, and under the mercury the greatest proportion of leaves to stems.

These relations are more evident in Table II which is a summary of all the data. In this table the average dry weight values obtained under the Mazda lamp are made equal to 1 and the ratio of dry weights produced under each of the other lamps has been calculated and placed in its respective place in the table. The figures show that if 1.0 gram of dry tissue is produced under the Mazda lamp, 1.10 grams would be produced under the neon lamp, 0.90 gram under the sodium and 0.66 gram under the mercury lamp at the same light intensity as indicated by the Weston

TABLE II

RATIOS OF DRY WEIGHT PRODUCED UNDER EACH LAMP AS COMPARED WITH THE MAZDA = 1.00; CALCULATED FROM TABLE I; WEIGHTS IN GRAMS PER PLANT

	Ratio leaf areas per plant	Stems	Leaves	Whole plant	Weight calculated to equal energy basis in the visible region*
Mazda	1.00	1.00	1.00	1.00	1.00
Sodium vapor	1.12	0.70	1.12	0.90	1.41
Neon	1.00	0.92	1.35	1.10	1.20
Mercury vapor	0.73	0.43	0.96	0.66	0.62

Total energy ratio at soil level: Mazda 1.00, neon 0.20, sodium 0.18, and mercury 0.18. Energy in gram calories per square centimeter per minute for Mazda = 0.40 to 0.42.

* The figures in this column represent the dry weight which might be obtained if the calculated equal energy values in the visible region had been used. For method of calculation see text.

photronic cell. The total energy ratios at the soil level are indicated below the table. In general the energy figures indicate that it takes at least five times as much total energy to produce one gram of dry weight under the

FIGURE 1. Buckwheat plants. A. Left to right: greenhouse, neon lamp, Mazda lamp. B. Greenhouse, sodium vapor lamp, and Mazda lamp. C. Greenhouse, mercury vapor lamp, and Mazda lamp.

Mazda as compared with the neon lamp. The table also shows that the amount of leaf tissue produced under each lamp was more nearly the same, being slightly higher under the neon and sodium and lower under the mercury. The figures in the second column of Table II on the ratio of leaf areas per plant are less accurate than the weight figures in column 4, since the areas are calculated from measurements on only three plants from each test. The greatest difference appears in the figures for dry weight of stems. The greatest stem weight was produced by the Mazda lamp; the least by the mercury; while the neon and sodium were intermediate in effect. The excess elongation of stems under the Mazda lamp is also apparent as shown in Figure 1 A, B, and C. The small amount of stem growth under the mercury lamp is shown in Figure 1 C. The values calculated to an equal energy basis in the visible region appearing in the last column of Table II are discussed under the following title.

WESTON CELL SENSITIVITY AS RELATED TO ENERGY OUTPUT OF THE VARIOUS LAMPS

It is generally admitted that only the visible region and probably the near ultra-violet are effective in photosynthesis, and therefore in dry weight production of plant tissue. A discussion of this is beyond the scope of this paper, but assuming it to be true, it is important to consider how accurately the Weston cell indicates energy in the visible and ultra-violet regions, and especially in the visible region since the energy emitted by the lamps in the ultra-violet is probably negligible in photosynthetic effect. The Weston cell sensitivity curve is reproduced in Figure 2 from data published by the manufacturers (7). In this same figure is reproduced the relative energy distribution of the Mazda lamp which corresponds to the black body radiation curve at 3000° K. This curve was supplied by F. A. Benford and L. C. Porter of the General Electric Company. The 500-watt Mazda lamp used in each comparison test with a neon, sodium, or mercury lamp was wound for a 110-volt current but was operated upon a 118- to 120-volt supply line. According to data published by Forsythe (6), the temperature of such a lamp approaches closely 3000° K. The energy data for the low pressure mercury arc lamp appearing in Table III were obtained from data published by Barnes (3) while those for the neon lamp were from the data of Benford and Buttolph (4). It was assumed that all the energy output of the sodium vapor lamp is at wave length 589mμ. This is not strictly true since sodium has lines at 568mμ and several weaker lines in the blue-violet region. The Weston cell has a high sensitivity at 568mμ and the energy in the weaker lines is extremely small, so that the first assumption appears correct. Some energy is found in the neon lines in the output from the sodium lamp. Neon is placed in the sodium tube by the manufacturer to start the discharge and these lines

are still present in the spectrum (Fig. 3, No. 6), when the lamp has reached the maximum operating temperature for sodium. It is believed that the small amount of energy present in the neon lines is negligible in photosynthetic effect. Using the values from this output data, the percentage of the total visible energy was calculated for each principal line in the visible region. The value found for each line was then multiplied by the corresponding percentage sensitivity of the cell at this particular wave length.

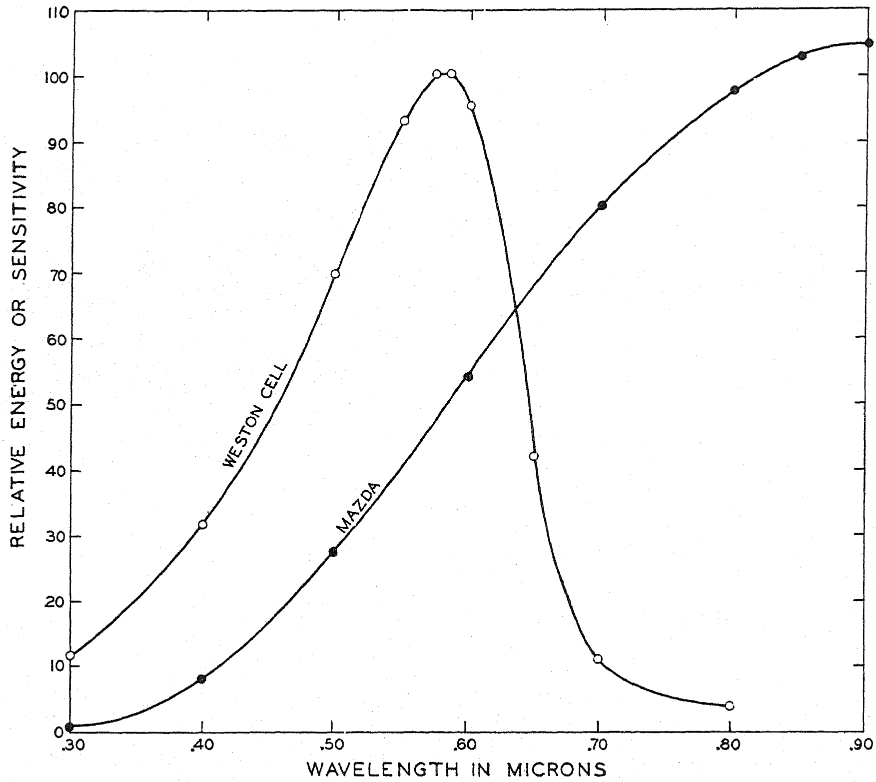

FIGURE 2. Sensitivity graph of Weston cell and relative energy output of 500-watt Mazda lamp at a filament temperature of 3000°K.

The values found for each wave length or line from 700 to 400mμ (700 to 365mμ for mercury) were then added to find what fraction of the total visible output of the lamp would register on the cell. Inthe case of the Mazda lamp the percentage energy at each 10mμ step was multiplied by the respective ordinate of cell sensitivity at this wave length as this lamp does not produce a line spectrum. The calculations show that 100 units in the visible region of the neon lamp register as only 65.4 on the Weston cell. For the Mazda, 100 energy units register as 62.4, and for the sodium

TABLE III

RELATIVE ENERGY PERCENTAGE OF NEON AND MERCURY VAPOR LAMPS AT VARIOUS WAVE LENGTHS AS COMPARED WITH SENSITIVITY OF THE WESTON PHOTRONIC CELL*

Wavelength in millimicrons	Weston cell sensitivity	Output neon % of total	Wavelength in millimicrons	Weston cell sensitivity	Output neon % of total	Output mercury % of total
672	0.27	0.81	609	0.90	4.95	—
667	0.34	1.70	607	0.92	3.31	—
660	0.40	1.20	603	0.93	1.30	—
653	0.42	1.01	597	0.96	0.96	—
651	0.42	3.99	595	0.96	3.84	—
640	0.53	11.02	588	0.98	2.37	—
638	0.56	3.30	585	0.98	3.92	—
634	0.60	3.90	577	0.98	—	9.40
630	0.68	1.22	575	0.98	0.59	—
627	0.73	3.43	546	0.91	—	26.40
622	0.80	1.28	540	0.90	0.54	—
616	0.86	2.50	435	0.44	—	13.20
614	0.87	8.27	405	0.33	—	6.90
			365	0.21	—	2.20

* Energy distribution for neon taken from data by Benford & Buttolph (4), and for mercury from data by Barnes (3).

lamp, 100 are equivalent to 98, while 100 energy units from the mercury lamp appear as 58.1 on the cell. When each lamp was adjusted in height to read 70 millivolts on the Weston cell at the soil level the following values of energy in the visible region are therefore calculated as having been attained: Mazda 112, neon 104.3, sodium vapor 71.4, and mercury vapor 120.4. From Table II, assuming that dry weight production increases or decreases in proportion as the intensity of each lamp increases or decreases, it may be calculated that if equal energy values for each lamp in the visible region had been used, the dry weight production per plant would have been as follows: Mazda 0.89 gram, neon 1.06, sodium 1.26, and mercury 0.55. Again adjusting these values to the dry weight produced under the Mazda as equal to 1 gram per plant (that is, dividing each value by 0.89), the resulting figures for the other lamps are as follows: neon 1.20, sodium vapor 1.41, and mercury vapor 0.62. It is unfortunate that suitable filters are not available which transmit very sharply only the visible region and absorb all infra-red. Such a filter could be used with a thermopile in place of the Weston cell to adjust each lamp to equality for each test directly and without further calculation. Nevertheless, these calculations from the data obtained in the tests indicate that the visible energy from a sodium lamp is slightly more effective in the dry weight production of plants than the neon, while the neon is more effective than Mazda, and the mercury vapor is the least effective. From this it follows that blue light is much less efficient in dry weight production than either the sodium line or the red-orange region emitted by the neon lamp. It also appears that the sodium line is more efficient than the longer wave lengths at the red end of the spectrum. The efficiency of the sodium line is even more interesting when it is recalled from data published by Buttolph (5) that the sodium lamp has an efficiency of 45 lumens per watt as compared with 22 lumens for the Mazda, 16 for the neon, and 15 for mercury. It has an additional advantage in the current consumption of the auxiliary which amounts to only 25 watts as compared with 200 watts in the arc itself. This same ratio for the neon lamp auxiliary to arc is 200 to 300 watts and for mercury it is 175 to 275 watts.

In general, these values are in agreement with those of Warburg and Negelein (9) for the alga *Chlorella.* They found blue light ($\lambda = 436 m\mu$) to have an average photosynthetic value of 33.8 as compared with a value of 53.5 for yellow ($\lambda = 578 m\mu$), a ratio of 1 to 1.58. The ratio of sodium light efficiency to mercury light from the data obtained above is 1 to 2.3. Warburg and Negelein, however, found red light ($\lambda = 660 m\mu$) to be 1.13 times as effective as yellow ($\lambda = 578 m\mu$). This does not agree even in direction with the results set forth above in which neon light was found to be less effective than sodium. Warburg and Negelein did not include the sodium line ($\lambda = 589 m\mu$) in their experiments. If there is a peak of efficiency in this

region ($\lambda = 589\text{m}\mu$) as the present data indicate, this would not have been brought out by the experiments conducted by Warburg and Negelein.

EFFICIENCY OF ENERGY BANDS IN THE VISIBLE REGION AS RELATED TO CHLOROPHYLL ABSORPTION

Zscheile (10) has recently published both graphic and tabular data showing the absorption of especially purified chlorophyll *A* and *B* from wave lengths 400 to 700mμ. The points of maximum absorption for chlorophyll *A* are at wave lengths 427.5 and 660mμ. The corresponding maxima

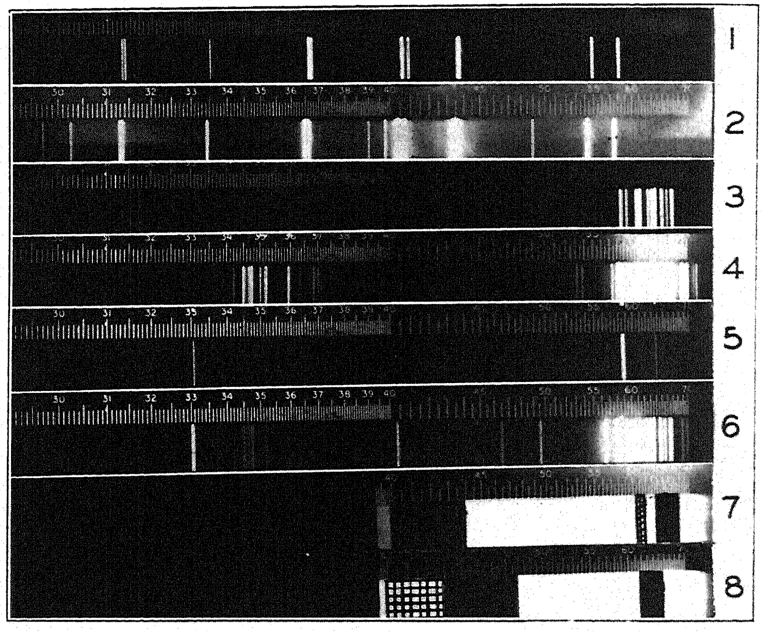

FIGURE 3. Emission spectra of lamps and absorption of chlorophyll. Nos. 1 and 2, mercury vapor lamp exposures 1× and 30×. Nos. 3 and 4, neon lamp exposures 1× and 30×. Nos. 5 and 6, sodium vapor lamp exposures 1× and 30×. No. 7, absorption of chlorophyll *A*. No. 8, absorption of chlorophyll *B*.

for *B* are at wave lengths 452.5 and 642.5mμ. The main emission lines for each gaseous discharge lamp and also the region of absorption for *A* and *B* chlorophyll are shown in Figure 3. The exposures are in pairs for each lamp, the second exposure of each pair being 30 times the first, and the first either 1 or 2 seconds. The main lines are therefore shown in the first exposure of each pair while the weaker lines which appear only after long exposure are shown in the second. A comparison of the emission lines of each lamp with the data of Zscheile and the absorption bands of chlorophyll as indicated in Figure 3, Nos. 7 and 8, shows that there is no

relationship between the amount of energy effective in dry weight production at a given wave length and the corresponding absorption value of chlorophyll. Roodenburg (8) found that the neon lamp was very effective for forcing plants and concluded that this was because the emission lines of neon correspond very closely with the main absorption band of chlorophyll in the red region. Similar reasoning would indicate that the sodium lamp with most of the energy in the sodium lines at 588 to 589mμ would be ineffective as both of these lines are outside of the main absorption band of chlorophyll. From the data of Zscheile, the absorption at wave length 588mμ is less than 10 per cent. The experimental data set forth above show that the sodium lamp is very effective, and from calculation on an equal energy basis in the visible region is more effective than neon. On the other hand mercury lines between wave lengths 365 and 480mμ in the blue-violet chlorophyll absorption band should be very effective, whereas they are less effective than any of the lamps tested. It may be concluded therefore that chlorophyll absorption has very little if any direct relation to the efficiency of a band of energy in the production of dry weight increase in buckwheat plants. The absorption of other associated pigments may be related and also play an important part in photosynthesis, but a discussion of the absorption of these pigments is beyond the present considerations.

COLOR AND APPEARANCE OF PLANTS GROWN UNDER VARIOUS LAMPS

The excessive stem growth under the Mazda lamp has been pointed out in the preceding discussion. None of the gaseous discharge lamps in the tests produced this effect. The least stem growth is produced by the mercury lamp. The outstanding difference between the Mazda lamp and the other lamps is the high total energy value, the ratio being approximately 5 to 1 at the soil level as shown in Table II. This high ratio is due mainly to the excess infra-red in proportion to the visible output of the Mazda lamp. Further studies have been made as to the region in the output of this lamp producing such excess stem growth and will be published in a later paper. A second striking difference is the dark green leaves of plants grown under the gaseous discharge lamps. The leaves grown under the Mazda lamp were pale green in comparison although they were a slightly darker green than those grown in the greenhouse under the low intensity winter sunlight. Further studies have been made on the spectral region producing this bleaching action on chlorophyll and the results will also be published at a later date.

CONCLUSION

The growth and dry weight production of buckwheat plants were studied using neon, mercury vapor, and sodium vapor lamps in comparison with a 500-watt Mazda lamp as light sources. The Weston photronic cell was used to determine the point of equal light intensity in each com-

parison test. Placing the average dry weight per plant produced under the Mazda lamp equal to 1 the values found for the other lamps were: neon 1.10, sodium vapor 0.90, and mercury vapor 0.66. Calculating the dry weights which might be produced if equal amounts of energy in the visible region had been used, these values are as follows: Mazda 1.00, sodium vapor 1.41, neon 1.20, and mercury vapor 0.62. A consideration of the absorption spectrum of chlorophyll shows no relation between the emission bands of the various lamps, the absorption bands of the chlorophyll pigments, and the efficiency of the lamp in producing dry weight of plant tissue. The sodium lamp was found most efficient, with the main output of energy at wave lengths 588 and 589mμ, a point at which chlorophyll absorption is near the minimum. The neon lamp was second in efficiency with the main output band near the maximum of chlorophyll absorption in the red-orange region. The mercury vapor lamp was least efficient yet has much of the energy output in the blue-violet region where chlorophyll absorption is maximum. The sodium lamp has an efficiency of 45 lumens per watt and a remarkably low power loss in the auxiliary transformer unit of only 25 watts as compared with a current consumption of 200 watts in the arc itself. It offers considerable promise as a cheaper and more efficient light source for growing plants.

All gaseous discharge lamps produced greener leaves and a lower ratio of stems to leaves than the Mazda lamp.

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A NEW TYPE OF INSULATED GREENHOUSE HEATED AND LIGHTED BY MAZDA LAMPS

JOHN M. ARTHUR AND L. C. PORTER[1]

In this region low sunlight intensity and short days during the winter months bring the growth and consequently the flowering of many plants almost to a standstill. There is a real need of a practical combination of artificial light for a few hours each night with the sunlight which is available during the daylight hours. Such a combination inherently possesses the possibility of forcing springtime growth and flowering in many varieties of plants during the darkest days of winter. The common artificial light source of our generation is the Mazda lamp. Approximately 90 per cent of the energy output of this lamp is heat. It is evident therefore that it offers possibilities as both a heat and light source in the greenhouse. In general, electrical energy is more costly than heating directly with steam arising from the burning of coal, so that it is even more important to reduce all heat losses to the minimum when considering electrical greenhouse heating as a practical application.

The ordinary greenhouse is not designed to operate efficiently as regards winter heating. Little thought has been given to the general design and pitch of roof structure which will admit the maximum amount of sunlight with the minimum exposure of glass at the time that plants need light most. The main trend in present greenhouse construction is to develop something which is pleasing to the eye with little regard to practical value in growing plants. The conventional design demands a gabled roof beautifully rounded at the eaves, so that half of the roof, regardless of which way it is faced, transmits no direct sunlight but only sky light; yet because of the high heat transmission of glazed areas, this dark half loses a great amount of heat. It is believed that such a high heat loss as is presented by this dark half of the ordinary greenhouse is in no way compensated by the additional amount of sky light transmitted. To make the case even worse, the modern designer is not satisfied with the high heat loss through a glazed surface but must overlap each glass like the shingles on a roof and purposely leave a great number of cracks which admit air freely. This custom has probably been handed down through several generations of designers as a sort of folk lore which was initiated probably by the first studies in plant physiology, indicating that plants must have fresh air. The practice certainly does not lend itself to either the conservation of heat or the addition of carbon dioxide gas within the house and probably is not justified on any other grounds since the average green-

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house man prefers to open air vents to admit only sufficient air to get rid of excess solar radiation during the daylight hours. For this reason, it appeared desirable to disregard entirely the conventional style of greenhouse construction and to develop a style which would be more practical from a heating and lighting standpoint using electrical energy.

The first thought which came to mind in designing a more efficient house which might be heated and at the same time intermittently lighted was to place the house wholly underground and depend entirely upon artificial light. There are two reasons why this was not considered practical. First, the Mazda lamp is not as good in quality of light as sunlight. Earlier studies have shown that continued illumination with this lamp results in injury on tomato and geranium plants (1), yet these plants grow well on an 18-hour day, 12 of which are sunlight. Second, sunlight, even though of low intensity, is almost always brighter than the artificial light which can be added in a practical way. From records of the New York Meteorological Observatory for December, 1934, the average sunlight intensity for the hour 10 to 11 a.m. during the entire month was 19 gram calories per square centimeter. Using the conversion factor developed by Kimball (2), this is equivalent to approximately 1900 foot candles. This intensity is approximately the same as the illumination from a 1000-watt lamp reflected down upon a surface 3 by 2.5 feet. Such a consideration of the electrical energy necessary to equal the average solar intensity during a comparatively dark month makes it evident that sunlight cannot be disregarded either from the point of view of relative costs or of quality of light.

After a careful consideration of these facts, a plan of a new type of greenhouse was drawn which appeared to eliminate some of the undesirable features of the ordinary house. The house was built at the Boyce Thompson Institute with labor and material furnished by this organization. The General Electric Company furnished and installed the electrical equipment, and cooperated with the Yonkers Electric Light and Power Company in furnishing current.

CONSTRUCTION OF GREENHOUSE

In general, the house was built similar to a large refrigerator. An end elevation photograph of the house is shown in Figure 1 A, and a sectional drawing is shown in Figure 2. The skeleton frame consisted of wooden upright pieces, floor joists, and rafters all 2 inches by 6 inches. The frame was covered both outside and inside with sheets of galvanized steel, No. 16 gauge, and all joints inside were lapped and soldered while the joints outside were lapped only, except those of the roof which were also soldered. The four walls, floor, and roof were filled with dry sawdust which was packed tightly by means of wooden pestles. In effect this gave a 6-inch

wall of insulating material on all sides, floor, and roof with the exception of a section of the roof and south side of the house occupied by the windows. The window section consisted of 8 separate, two-light, ordinary storm sash hinged at the top. Each sash was 28 inches wide and 6 feet long,

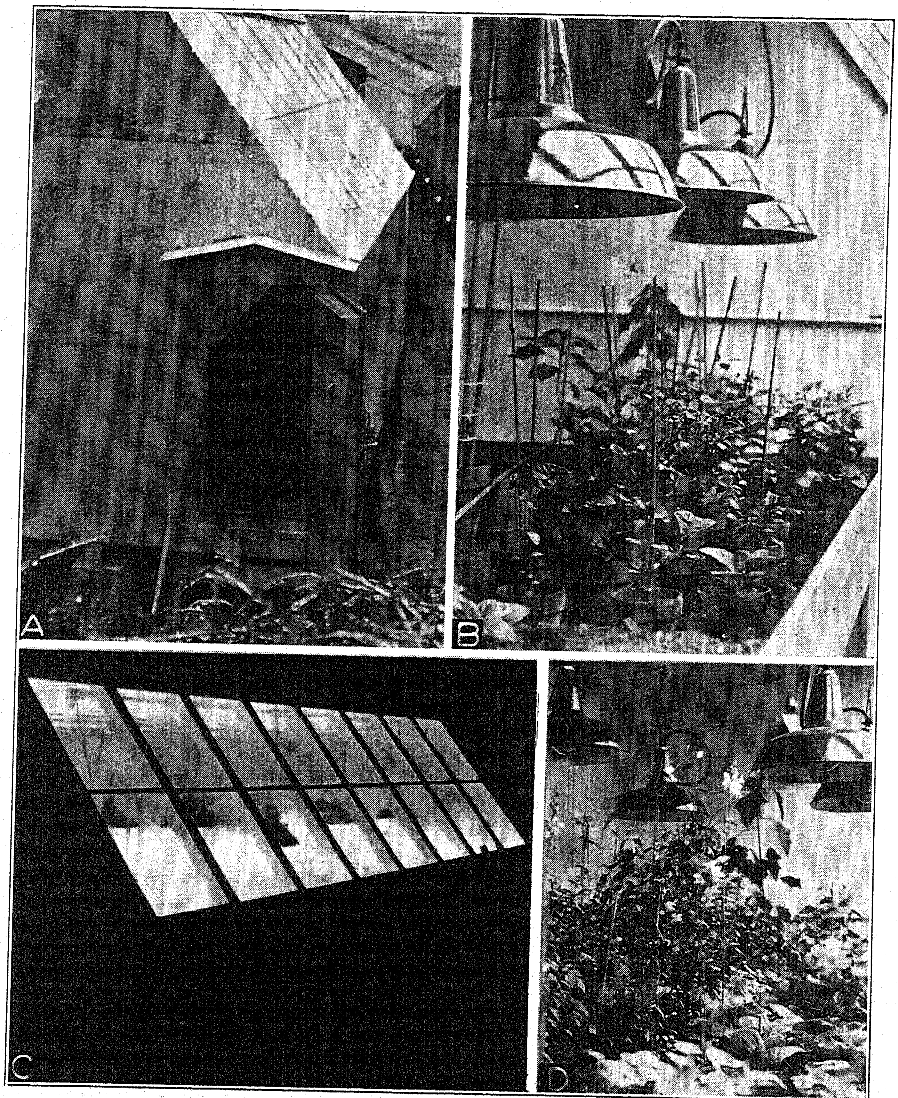

FIGURE 1. (A) An end view photograph of the new type greenhouse showing the refrigerator type of door and the method of supporting the house on cement pillars. (C) A night view through the storm sash. (B) A photograph of the plants taken inside the house on December 29. (D) A photograph of the same plants taken February 9, or 42 days later.

glazed with two glasses each 24 inches by 32 inches. The glasses were set in the wooden frames, imbedded in putty and paint so as to form air-tight joints. Although double glazing would result in less heat loss, the decrease in sunlight transmitted by another layer of glass would no doubt more than offset any advantage gained, and it was found by actual operation of the house that single glazing furnished sufficient insulation. The glazed section can be seen in the night photograph reproduced as Figure

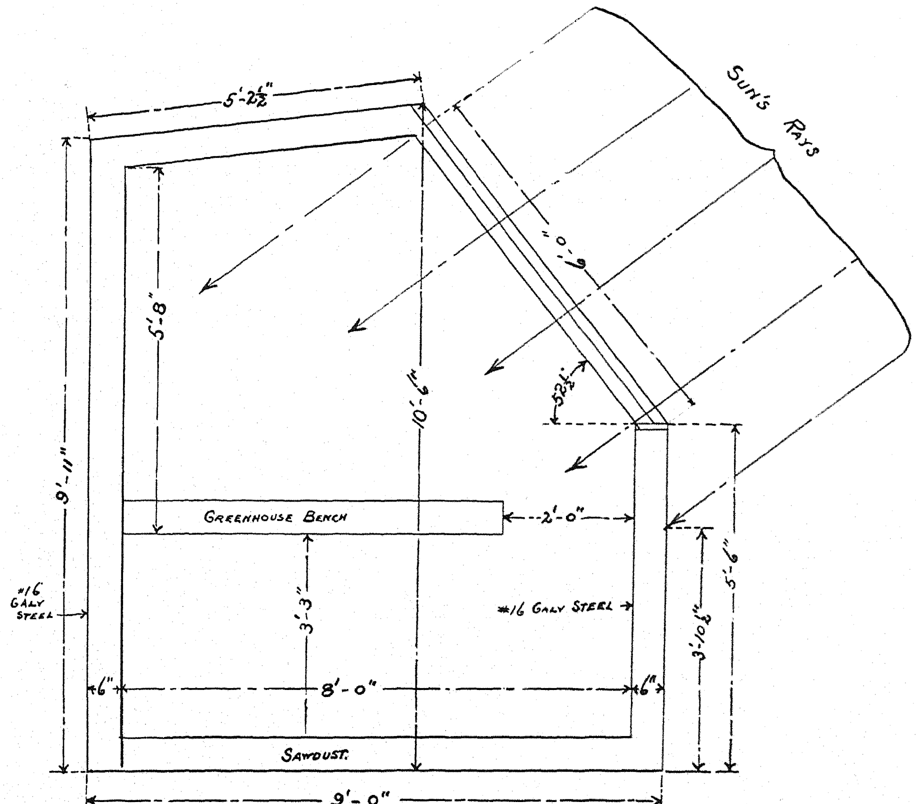

FIGURE 2. Sectional drawing of house giving structural dimensions.

1 C. The single door to the house was of the refrigerator type with six inches of sawdust between the inner and outer face. It was purposely made small, 2 by 4-1/2 feet, to guard against excess heat losses when opened. In order to admit as much sunlight as possible during the winter months when the intensity is lowest, the glazed sash were pitched at an angle of approximately 52-1/2° to the horizontal or water level. This angle was chosen because it places the sash perpendicular to the incident rays of sunlight at noon at a point half way between the autumnal equinox and winter solstice and again between the winter solstice and vernal

equinox, that is, on about November 5 and Feburary 5. It may be calculated for any location by adding the latitude to 102° and subtracting this value from 180°. This gives the approximate altitude of the sun above the horizon on these dates. The angle of the sash is found by subtracting this value from 90°. It should be pointed out that this angle is especially desirable during the months of October, November, December, January, and February. By the latter part of March in this latitude the sun will reach a sufficient altitude to cast a shadow of the front of the roof on the back part of the growing bench at noon. This shadow can be avoided either by placing the bench against the south wall of the house and placing the walk and the entrance door along the back or north wall, or by tilting the house backward.

The single, six-foot growing bench was placed along the back wall in the house as now constructed, leaving a walk two feet wide between the bench and the front or south wall. The inner walls above the bench were painted white so as to reflect as much light as possible down upon the growing plants.

Two rows of five lamps each, extending along the entire length of the house were used as a source of both light and heat. During December, January, and the first 3 weeks of February, all 500-watt lamps were used. The lamps were spaced on centers 3 by 3-1/2 feet, leaving 1-1/2 feet of space between the back wall and the first row of lamps. This arrangement gave a very uniform intensity over the whole bench. Each lamp was counterweighted separately by means of sash weights and pulleys so that it could be raised or lowered to accommodate the growth of plants in each part of the growing bench. All lamps were operated by means of a single thermostat suspended from the roof over the center of the growing bench. This thermostat operated an electro-magnetic switch located at one end of the house above the growing bench which turned the lights on when a certain minimum temperature was reached and operated again in the reverse direction when a certain maximum was reached. This operation will be discussed later. Each lamp was equipped with a standard R.L.M. reflector as illustrated in Figure 1 B and 1 D.

PLANTS GROWN

Since the house was not completed until the latter part of November, the planting for last year was considerably delayed. If it is desired to grow cut flowers for the Christmas season, it would be necessary to start the plants about a month earlier. All plants were started in the house on December 10. In most cases small plants about 2 inches high were used. These had been grown in another greenhouse either in flats or small pots. One-half of the bed in the new type house was filled with soil to a depth of 6 inches and all of the plants planted in this half were removed from

pots and plunged directly into the soil. The other half of the bed was filled only to a depth of 2 inches and the plants were grown in pots placed directly upon this shallow layer of soil. It was observed during the tests that the plants in pots grew more rapidly and were more resistant to wilts and other diseases favored by high humidity. In order to further decrease the humidity it is desirable to use only pot-grown plants and no beds of soil in the new type house.

The following species of plants were chosen and grown as being representative flowering plants grown commercially in greenhouses during the winter season: *Senecio cruentus* DC. (cineraria, giant exhibition), *Lathyrus odoratus* L. (sweet peas, Burpee #3300), *Antirrhinum majus* L. (snapdragon, giant mixed), *Euphorbia pulcherrima* Willd. (poinsettia), *Solanum pseudocapsicum* (Jerusalem cherry), *Calceolaria* sp. (hybrids), *Begonia semperflorens* Link & Otto, *Fuchsia hybrida* Voss, and *Gardenia jasminoides* Ellis. In addition *Fagopyrum esculentum* Moench. (buckwheat) was grown in both the insulated and control houses in order to determine the relative dry weight of plant tissue which may be produced. This plant has been found to increase in dry weight with increasing light intensity and increasing day length, and since it flowers on all day lengths, it furnishes a biological indicator of the efficiency of light sources in dry weight production of plant tissue when temperature and other factors are comparable. The buckwheat plants were grown from seed and were then cut down, dried, and weighed just as the flowering stage was reached. The plants were grown in pots averaging ten seedlings per pot. The first crop was harvested on January 25 from seed which had been planted December 10. One pot of plants marked (−) from the ordinary greenhouse and one pot marked (+) from the insulated house are shown in Figure 3 A. The weights were as shown in Table I A. It will be seen that the new type of

TABLE I

AVERAGE DRY WEIGHTS OF BUCKWHEAT PLANTS IN GRAMS PER PLANT

A. Grown from December 10 to January 25

	Pot numbers				Average
	1	2	3	4	
Ordinary greenhouse	0.22	0.22	—	—	0.22
Insulated greenhouse	0.69	0.72	0.75	0.72	0.72

B. Grown from January 25 to March 20

	Pot numbers			Average
	1	2	3	
Ordinary greenhouse	1.50	1.52	—	1.51
Insulated greenhouse	1.92	1.82	2.06	1.93

FIGURE 3. (A) Buckwheat, January 25, from ordinary greenhouse at left and from new type house at right. (B) Poinsettia, February 21, a plant from each house. (C) Sweet peas, January 16. (D) February 21, a plant taken from each house on each date.

insulated greenhouse is more than three times as efficient in building up plant tissue as the ordinary greenhouse during the two winter months, December and January. The second crop of buckwheat was planted January 25 and harvested March 20. The weights were as indicated in Table I B. There is much less difference in the dry weight of tissue produced in the two houses during the months of February and March. This is to be expected as both the natural day length and light intensity are increasing

FIGURE 4. (A) *Begonia*, one plant from each house on January 16. (B) Two plants from each house on February 21. (C) *Fuchsia*, one plant from each house on January 16. (D) The same on February 21. Plants from new type house at right in each case.

rapidly in the ordinary greenhouse, while the number of hours of artificial light in the insulated house is decreasing due to higher outside temperatures and longer solar day length. These factors tend to equalize the dry weight produced in the two houses.

With the longer day in the insulated house, the plants were brought into flower in a short time. Fuchsias and snapdragons were brought into flower in the insulated house during the last week in January. Fuchsias came into flower in the control house during the second week in February,

while snapdragons did not flower in this house until the last week in March. *Begonia* flowered in both houses but produced much larger plants in the insulated house. The first flower of *Calceolaria* opened in the new house on January 24 while the first flower appeared in the control one

FIGURE 5. (A) Cineraria, February 21, showing flowering in the ordinary greenhouse only at the left. (B) *Calceolaria*, January 16, two plants at left, ordinary or control greenhouse, and two at right from the new type house. (C) The same plants on February 21. (D) Snapdragon, January 16. Two at left, control, and two at right, from new type house. (E) The same plants except three from the new type house at the right on February 21. (F) Jerusalem cherry, two plants from each house on January 16. (G) The same plants on February 21.

month later. Sweet peas flowered two weeks earlier in the insulated house. The only plants which flowered first in the control were cineraria and

poinsettia. These are apparently short day plants and the long days produced by the additional light each night completely inhibited flowering. The cinerarias came into flower in the control house during the second week of Feburary. Out of ten plants in the insulated house, only one, at the back of the house, which was shaded by taller plants, produced a flower. Poinsettia grew to more than three times the original height in the insulated house but did not flower, while it flowered but did not grow appreciably in the control. In the practical operation of such a greenhouse, it is therefore essential to avoid those varieties of plants which flower only on short days. In general, these are the plants which start flowering under natural conditions in August or September as the days become shorter. Both the growth rate and flowering of Jerusalem cherry were more than doubled in the insulated house. No fruits were set as the flowers were not pollinated. Photographs reproduced as Figures 1 B, taken December 29 in the new house, and 1 D, taken in the same place on February 9, illustrate the rate of development of the plants. During this short period of 42 days all species except the two short day plants already mentioned came into flower. In order to show the relative growth of plants in the ordinary greenhouse as compared with the new type of house, representative plants were photographed at two stages of development, on January 16 and again on February 21, and are reproduced as Figures 3, 4, and 5. In each group the plants from the ordinary greenhouse are on the left and are marked with a minus sign (−), while those from the insulated house are on the right and are marked with a positive (+) sign. The date on which each photograph was taken is indicated in the respective legend for each figure. It should also be pointed out that the plants were transferred into larger pots as the increase in size demanded. The (−) and (+) pots of plants in each group are photographed to the same scale.

TEMPERATURE CONTROL AND OPERATION OF THE THERMOSTAT IN THE NEW TYPE HOUSE

The thermostat was first set to hold a temperature between 72° and 78° F. It was soon found that the plants grew too rapidly at this high temperature, although it was not too high for seed germination. The setting was then changed to a range of 62° to 68° F., a temperature which was found to produce a much more desirable type of growth. The Mazda lamp at higher temperatures has a tendency to produce excess elongation of plants but this is largely counteracted by maintaining a low air temperature, and, with the balancing effect of sunlight during the day, works remarkably well for forcing plant growth. The temperature ranges mentioned above were obtained from an electrical resistance recording thermometer placed above the plants near the roof and toward the back wall of the house. Mercury thermometers hung along the south wall above the

walk indicated a temperature 8° to 10° lower. The records above therefore may be considered as representing the warmest part of the house but they also agree closely with the air temperature among the growing plants. The range of the thermostat at the start was 6°. It was found that as the lamps were raised to keep above the tips of the rapidly growing plants, more light fell upon the instrument from the two nearest lamps. This shortened the range to 4°, and this fact, discovered by observation of the experiment, was used to further reduce the range. During the last few weeks of growth, a small 50-watt lamp was connected to the lamp circuit and was placed approximately 6 inches back of the thermostat. This narrowed the range to 1°, and for several weeks the temperature in the house

TABLE II

RELATION OF OUTSIDE AIR TEMPERATURE TO TIME AND AMOUNT OF ILLUMINATION

Date	Mean temp., °F.	Max. temp., °F.	Min. temp., °F.	Total current used, K.W.H.	Times on, lamps	Average dark period in mins.	Average lights on, time mins.	Total hrs. of burning
9 A.M. Jan. 27 to 9 A.M. Jan. 28	2.9	13	−1	26	29	33	10.0	5
9 A.M. Feb. 5 to 9 A.M. Feb. 6*	13.7	18	6	32	54	20	7.1	6.4
9 A.M. Feb. 27 to 9 A.M. Feb. 28	16.7	23	14	20	64	14	6.2	6.6

* The figures are for the entire 24-hour period. This was a dark day and the lamps burned 15 times during the day, and 39 times during the night. The other two cases represent only current consumption during the night as the lamps were not on during the day.

each night was held within this narrow range of $\pm\frac{1}{2}$° F. This arrangement of allowing the heat of the lamp to fall directly upon the thermostat greatly increased its sensitivity by decreasing the time required for heating the air immediately surrounding the thermostat as well as the instrument itself to a point at which it would again break the circuit. These changes in the range had a corresponding effect on the number of times the lamps came on during the night as well as on the time that the lamps burned before they were automatically turned off again by the rising temperature. To illustrate these points, a few typical cases will be discussed. A summary of the data is presented in Table II.

Nine a.m. January 27 to 9 a.m. January 28, 1935. The New York Observatory records taken at Central Park indicate a mean of 2.9° F. for this period with a maximum on the 27th at 4 p.m. of 13° F., and a minimum

of $-1°$ F. on the 28th at 4 to 6 a.m. The records at the Institute showed approximately this same temperature range. January 27 was a clear day and the lamps did not come on during the daytime, although the temperature inside the house reached 100° F. and the windows had to be opened to eliminate some of the solar heat. This was invariably the case, that whenever the sun was shining no current was used in lighting and heating the house, but instead the opposite problem of getting rid of the excess solar heat immediately arose. As the energy of sunlight fell away toward evening, the temperature inside of the house dropped rapidly, the windows were closed at 4:30 p.m., and at 5 p.m. the lights came on for the first time. The temperature had reached 60° F. The lamps burned until a temperature of 66° F. had been reached, approximately ten minutes, at which time the thermostat and the circuit breaker operated. From 5 p.m. until 9 a.m. the lamps came on 29 times, an average of once every 33 minutes, and consumed approximately 26 kilowatt hours of current during the entire night, or 0.89 K.W.H. each time the lamps burned. This arrangement adds therefore approximately five hours to the natural solar day length which the plants receive.

Nine a.m. February 5 to 9 a.m. February 6, 1935. The New York Observatory records show a mean of 13.7° F. for this period with a maximum of 18° F. and a minimum of 6° F. February 5 was a dark day—one of the six darkest days in the entire month. The lamps were on 15 times during the day from 9 a.m. to 5 p.m., consuming 7 K.W.H. From 5 p.m. until 5 a.m. 25 K.W.H. were used and the lamps were on 39 times. During this time, the lamps had been raised so that the range of the thermostat was shortened to 4° F. (from 60° to 64°). The current used each time the lamps burned during the night was approximately 0.64 K.W.H. or the lamps were on slightly less than 7.7 minutes each time, while during the day the corresponding figures are 0.47 K.W.H. and 5.7 minutes.

Nine a.m. February 27 to 9 a.m. February 28, 1935. The Observatory records show a mean of 16.7° for this period, a maximum of 23°, and a minimum of 14° F. The day was clear except during the first hour of the morning when a local cloud brought the lights on twice; otherwise no current was consumed until 6 p.m., when the thermostat again started to operate. During this period the thermostat was illuminated when the lights were on by a 50-watt lamp fitted with a conical reflector placed at a distance of approximately 6 inches. This narrowed the range of the thermostat to 1° F. The lamps came on 64 times during the night and used a total of 20 K.W.H. It should be pointed out that the wattage of the lamps had been changed shortly before this observation. On February 20, all 500-watt lamps were removed and 300-watt lamps used as replacements. This indicates that the lamps came on as an average, every 14 minutes and burned for 6.2 minutes, or a total of 6.6 hours during the night.

COST OF OPERATION

Daily records were made of the current used in kilowatt hours. These data are presented in Table III. The average daily current consumption for each period following a change in the setting of the thermostat is shown together with similar data for the entire month and the entire period from December 6 to March 31. At each lower setting of the thermostat there is a corresponding decrease in the daily current consumption. Also as the intensity of sunlight increases in February and March, the current consumption falls off rapidly. This decrease is due both to the higher outdoor temperatures and the increase in the number of hours of sunshine or day length. The number of hours of artificial light can be increased by putting in smaller wattage lamps, but this increase is at the sacrifice of intensity in the artificial light supplied. Calculated from the current consumed during the months of December, January, and February, the average amount of artificial light added to the natural day length is four hours per day.

TABLE III
AVERAGE CURRENT CONSUMPTION AT VARIOUS MONTHLY PERIODS

Dates and temperature ranges of thermostat	Total current consumption, kilowatt hours	Average current consumed per 24 hour day—KWH
Dec. 6–18, 72°–80° F.	324	25
Dec. 19–26, 64°–72° F.	185	23
Dec. 27–31, 60°–68° F.	93	18
Dec. 6–31, ranges as above	602	23
Jan. 1–31, 60°–68° F.	620	20
Feb. 1–20, 60°–68° F.	348	17
Feb. 21–23, 300-watt lamps, 60°–66° F.	52	17
Feb. 24–28, 300-watt lamps and 50-watt lamps near thermostat, 61°–62° F.	80	16
Feb. 1–28, ranges as above	481	18
March 1–31, 61°–62° F.	321	10
Entire period, Dec. 6–March 31	2024	18

It is especially interesting that a greenhouse 19×8 feet inside dimensions can be heated and lighted during the coldest winter months at an average current consumption of 18 K.W.H. per day. At a cost of two cents per kilowatt, which is probably near the average cost for power in the United States, this amounts to only 36 cents per day. Figures supplied by a greenhouse manufacturing company indicate that approximately three tons of hard coal would be required to heat an ordinary greenhouse of these dimensions during the season. Assuming the cost of coal to be $8.00 per ton, it would cost approximately $20.00 for coal for this period or at an average rate of 17 cents per day. Although the electric cost of heating is more than twice as much as the cost of coal for steam heating,

the difference is more than compensated by the greatly increased growth and flower production in case of the electrical method of heating. In addition, a considerable cost for labor of firing the steam boiler must be added to the fuel costs, while the electrical method of heating requires no attendants.

COST OF CONSTRUCTION

The cost of all material entering into the construction of the house except the electrical wiring, thermostat, electro-magnetic switch, lamps, and reflectors, was approximately $350.00. This includes the cost of 4360 pounds of sawdust used as an insulating material which amounted to almost 12 per cent of the total cost of the building. The above figures do not include labor for construction, cost of cement, or other foundation material. In contrast with the above, the cost for fabricated material only for the standard type of ridge-roof greenhouse of similar dimensions is estimated at $500.00.

The cost of the electro-magnetic switch and thermostat used in the thermostatic operation of the lamps was $43.00, while the cost of the 10 lamps with reflectors is approximately $69.00. Since the cost of the wiring and labor for installing the electrical equipment varies with the locality, these cost figures will not be discussed here.

VOLTAGE OF LAMPS USED

In these tests 110-volt, 500-watt lamps were used at the start. These were burned on a 120-volt supply line. This arrangement produces a higher filament temperature, more light, and less heat than the usual practice of burning lamps at their labeled voltage, but results in a much shorter life of the lamps. The lights were first turned on during the day of December 6, although the plants were not placed in the house until December 10. Fifty per cent of the lamps failed and had to be replaced by February 10 at a meter reading of 1441 K.W.H., indicating an average life of approximately 290 hours. In contrast, a 120-volt lamp burned on a 120-volt line has an average life of 1000 hours. It is believed that a 115-volt lamp burned on a 120-volt supply line offers a better compromise between the present cost of lamps and cost of current, but at the same time, it should be stated that both the quality and intensity of light is slightly improved when the lamp is operated at a higher filament temperature.

METHOD OF INCREASING CARBON DIOXIDE CONCENTRATION

During the tests reported herewith, a lump of solid carbon dioxide with an average weight of 40 pounds was placed in a sheet metal double-walled box located inside of the greenhouse at 10 to 14-day intervals. The box had a 3-inch layer of powdered cork between the walls to serve as insulating material. The metal cover of the box was sealed with modeling clay

to form an air-tight joint. A small metal tube led out from the interior of the box and extended along the growing bench at a height of 3 or 4 inches above the soil. This tube was perforated at 6-inch intervals and served to conduct the carbon dioxide arising from the evaporation of the Dry-ice to the growing plants. It is not known how much additional growth was produced by the carbon dioxide treatment. It is thought from previous studies, that greater flowering is obtained by this treatment but the amount of the increase could only be determined by another similar greenhouse operated in the same way except with no additional gas. The writers are indebted to the Michigan Alkali Company for furnishing the Dry-ice for these tests.

DISCUSSION OF RESULTS

The new type of insulated greenhouse is believed to offer many advantages both to the ordinary householder, who wishes to grow a few of his favorite species of plants for cut flowers during the winter months, and to the commercial grower who wishes to force flower production rapidly for special holiday seasons. It is designed to use heat economically and this same low heat loss method of construction greatly restricts gaseous exchange with the outside air and greatly favors the holding of higher concentrations of carbon dioxide. This same construction, however, results in a higher humidity than is found in the ordinary greenhouse. Many species are favored by high humidity while others which are especially susceptible to certain wilt diseases may be injured by it. The humidity may be held down to a certain extent by growing plants in pots instead of in beds of soil and by restricting the water supplied to the plants. When the sun is shining, it is necessary to keep some of the windows open slightly even though the outside temperature is near 0° F., so that the higher humidity obtains only at night and on cloudy days when the windows are closed. The water requirements of plants in a house of this type are much less than in the ordinary greenhouse. It is sufficient to water the plants very slightly once each day. The use of Mazda lamps for both heating and lighting works especially well since the lamps burn only on cloudy days and at night when more light is needed by the growing plants. During the late fall and early spring when outdoor air temperatures are high less light is used to heat the house, and in general less light is required by the plants than during the months of extreme cold. It has been found in previous studies (1) that daylight supplemented by four to six hours of artificial light each night, using Mazda lamps, produces the most rapid growth and development of a number of plants. When Mazda lamps are used, thermostatically controlled, to heat this type of greenhouse, it is found that the total additional light received each night amounts to approximately four hours, so that in practice the ideal amount of light is

supplied. While this supplementary light is supplied intermittently, it is further observed that in practice it produces much more rapid growth and flowering than is found in the ordinary greenhouse during the winter months. It is believed that the growth rate which obtains in an ordinary greenhouse in March and April can be duplicated in the new type of house during the months of December and January. When plants are supplied additional light, it is considered important to supply additional carbon dioxide at the same time so that photosynthesis may proceed at the maximum rate. The new type house is well adapted to the use of higher concentrations of this gas, since the rate of gaseous exchange through the walls and roof is extremely low as compared with the ordinary greenhouse.

SUMMARY

1. A new type of insulated greenhouse was designed and built which uses sunlight and Mazda lamps as the sole sources of both heat and light. The lamps were operated by means of a thermostat set to maintain a temperature above 60° F.

2. Using ten 500-watt Mazda lamps it was found that this house could be heated and lighted at an average daily current consumption of 18 K.W.H. during the winter season. Calculated at 2 cents per kilowatt, the average cost is 36 cents per day.

3. The average daily amount of light supplied to the plants as a supplement to daylight was four hours. Except on cloudy days all of this amount of light was supplied at night. This produced a rate of growth and flowering during the winter months when light intensity is lowest which was believed equivalent to that in an ordinary greenhouse in March and April.

4. The greenhouse was built with double sheet metal walls soldered at the joints and filled in between the metal faces with sawdust. This type of construction is almost air-tight so that additional concentrations of carbon dioxide gas may be maintained economically by the use of pieces of solid carbon dioxide.

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ANAESTHETIC PROPERTIES OF CARBON MONOXIDE AND OTHER GASES IN RELATION TO PLANTS, INSECTS, AND CENTIPEDES

P. W. ZIMMERMAN

The question of what constitutes anaesthesia and the possibility of anaesthetizing plants were renewed in 1932 in a publication on the effect of carbon monoxide on plants (3). Low concentrations of this gas caused responses which were thought to indicate anaesthesia in plants. *Mimosa pudica* in 0.25 per cent carbon ~~dioxide~~ became insensitive to external mon stimuli and lost its capacity to assume the normal position of the leaves. The possibility that carbon monoxide might properly be classed as an anaesthetic was further tested by subjecting insects and other invertebrate animals to high concentrations of the gas, thereby causing loss of irritability. Eighty to ninety per cent of carbon monoxide in air was necessary to cause anaesthesia. With this high concentration of the anaesthetic, it was suggested that the low oxygen supply might be a limiting factor. Since the first publication appeared, numerous other experiments with plants and animals were conducted using carbon monoxide, propylene, ethylene, butylene, or carbon dioxide, mixed with air or known amounts of oxygen and other gases. The results reported in the present paper verify the earlier assumptions and extend the present knowledge concerning the anaesthetic properties of several chemicals in relation to plants, insects, and centipedes.

MATERIALS AND METHODS

With the exception of carbon monoxide, the gases used in the experiments were purchased from Union Carbide and Carbon Corporation or its subsidiaries. According to the analyses furnished by the companies, the purity of the gases was as follows: ethylene 97.2 per cent, acetylene 99.8 per cent, propylene 97.3 per cent, butylene 86.6 per cent, and carbon dioxide 99 per cent. The carbon monoxide gas was made in the laboratory by heating 100 grams of oxalic acid with 300 cc. of concentrated sulphuric acid. After passing over soda lime and bubbling through potassium hydroxide to remove the carbon dioxide, the gas was collected over water as it displaced water in large inverted bottles. An analysis of the final product with which most of the results were obtained indicated 96 per cent carbon monoxide.

To obtain mixtures of the anaesthetics and oxygen, various proportions of water were displaced from graduated, smooth-lipped cylinders or large test tubes.

The following plants were used for the experiments reported in this paper: sweet pea (*Lathyrus odoratus* L.), cucumber (*Cucumis sativus* L.),

heliotrope (*Heliotropium peruvianum* L.), tobacco (*Nicotiana tabacum* L. var. Turkish), and sensitive plant (*Mimosa pudica* L.).

The following adult animals were used: rose chafer (*Macrodactylus subspinosus* Fabr.), katydid (*Microcentrum rhombifolium* Sauss.), centipede (*Bothropolys* sp.), honeybee (*Apis mellifica* L.), bumblebee (*Bombus* sp.), yellow jacket (*Vespa* sp.), praying mantis (*Paratenodera sinensis* Sauss.), grasshopper (*Melanoplus femur-rubrum* DeGeer), meadow grasshopper (*Orchelimum* sp.), and cockroach (*Blattella germanica* L.).

For the most part the animals were immersed in water and then moved up into the cylinder containing the gas mixture. Animals, like bees, which were hard to handle or might have been injured from being immersed in water were placed in pith capsules while being inserted so as to keep them dry.

During the course of the investigations 325 insects were used. The number employed in the different experiments varied from one to four. After it was evident that there was very little difference in response of the individuals to the gases one animal was generally used in each experiment. When the critical concentration of gas for complete anaesthesia was approached the experiments were repeated three or more times. For example, 80 per cent carbon monoxide plus 13.8 per cent oxygen did not appear to completely anaesthetize centipedes, but 81.5 per cent carbon monoxide plus 13.6 per cent oxygen rendered the animal insensible within one minute. The experiment was then repeated three times with the latter mixture using one centipede each time.

Insects and centipedes were considered to be anaesthetized when they did not move, failed to right themselves when turned on their backs, and were insensible to touch. They were thought to be partially anaesthetized when they were made sluggish, yet responded mildly to touch or attempted to right themselves when abnormally oriented.

Mimosa pudica, the sensitive plant, was thought to be anaesthetized when the leaves failed to respond to external stimuli as touch or the heat from the flame of a match. With exposure to low concentrations of the gases the plants responded abnormally to external stimuli, that is, slowly or partially compared to controls. Under the latter conditions the sensitive plants were considered to be partially anaesthetized.

Other plants which do not respond readily to touch or the heat from a flame were judged by their growth and growth movements as shown by motion pictures and retardation in the elongation of the stem. When the concentration of the gas was high enough to cause pronounced epinasty of the leaves, the movements, due to irregular growth in different parts of the stem, varied from the normal. As the concentration of the gas increased the rate of elongation of the stem decreased and therefore growth movements were less, finally ceasing. Under such conditions the plant was con-

considered anaesthetized. It might be said that the anaesthetic produced growth rigor.

RESULTS

In order to determine whether a decreased oxygen supply in the gas mixtures was a limiting factor, invertebrate animals were observed while being exposed to nitrogen and helium mixed with small amounts of oxygen. As is shown by Table I, no ill effects resulted from exposure of the animals to 98 per cent nitrogen or helium when there was 2 per cent oxygen present. The containers used in the experiments were large enough to permit of 21 cc. of oxygen when only 2 per cent was present. Thus the total amount of oxygen was large enough not to be a limiting factor though the exposure of the animals to a given mixture lasted for several hours. When carbon monoxide and other gases were tested precautions were taken to have an ample supply of oxygen present.

TABLE I

RESULTS OF EXPOSURE OF CENTIPEDES AND ROSE CHAFERS TO NITROGEN, HELIUM, AND OXYGEN

Animal	Per cent of gases used			Duration of exposure	Effects
	N_2	He	O_2		
Centipede	98		2	1 hr., 50 min.	None
	100		0	1 hr.	Rendered insensible
			100	24 hr.	None
		95	5	35 min.	None
Rose chafer		95	5	30 min.	None
		100	0	15 min.	Rendered insensible
			100	20 min.	None

The results obtained with the methods described proved that it was possible to anaesthetize insects and centipedes with carbon monoxide gas. Centipedes were anaesthetized with 81.5 per cent carbon monoxide when the oxygen supply was 13.6 per cent. Eighty per cent caused the centipedes to become somewhat sluggish but did not completely anaesthetize them. The longest period of exposure given was 46-1/2 hours in a mixture of 80 per cent carbon monoxide and 15 per cent oxygen. This severe treatment caused the animals to become sluggish but they recovered readily when placed again in normal atmosphere. Carbon monoxide was much less toxic than ether or chloroform which are among the oldest known anaesthetics. Exposure to small amounts of these gases for an hour or less killed the animals. The lowest concentrations of the various gases found to anaesthetize insects and centipedes are shown in Table II.

The different species of animals tested varied in their resistance to carbon monoxide. To anaesthetize rose chafers it was necessary to use 85 per cent carbon monoxide and for katydid 89 per cent. Rose chafers re-

TABLE II

LOWEST CONCENTRATION OF VARIOUS GASES MIXED WITH OXYGEN FOUND TO ANAESTHETIZE INSECTS AND CENTIPEDES

Animals	Per cent of anaesthetic	Per cent of oxygen in mixture	Duration of exposure in minutes	Seconds required to anaesthetize	Time for recovery after remova
			Propylene		
Centipede	30	70	30	60	5 sec.
Katydid	75	25	35	10	5 min.
Rose chafer	60	40	30	300	5 min.
			Carbon monoxide		
Centipede	81.5	13.6	15	60	5 sec.
Katydid	89	5	30	1	10 min.
Rose chafer	85	8.4	30	120	2 min.
			Butylene		
Centipede	5	95	15	300	1 sec.
Katydid	10	90	30	180	30 min.
Rose chafer	40	60	15	10	30 min.
			Carbon dioxide		
Rose chafer	30	70	20	25	1 sec.
			Acetylene		
Rose chafer	100	0	15	20	15 min.
			Ethylene		
Centipede	100	0	50	15	3 min.
Katydid	100	0	30	5	15 min.
Rose chafer	100	0	20	15	15 min.
Rose chafer	Ether	Air	10	20	1 hr.
Rose chafer	Chloroform	Air	10	10	Dead

mained anaesthetized in an atmosphere containing 85 per cent carbon monoxide for 16-1/2 hours without serious effects. After removal from the gas they made complete recovery within five hours.

Propylene was a very effective anaesthetic, 30 per cent being sufficient to anaesthetize centipedes. No bad effects were observed from exposure to 40 per cent for 2-1/2 hours. The katydids could not be anaesthetized with 50 per cent propylene but were rendered insensible in 75 per cent with ten seconds' exposure. Rose chafers were anaesthetized with 60 per cent propylene.

Butylene was the most potent of all the unsaturated chemicals tested. The following concentrations were found to completely anaesthetize the animals: centipede 5 per cent, katydid 10 per cent, and rose chafer 40 per

cent. It should be noted that this is not the same order for effectiveness of propylene listed above. Long exposures to high concentrations of butylene were lethal. Paralysis of the legs followed complete anaesthesia. When removed from the butylene, the insects showed signs of recovery first by movement of antennae and next the front legs. After exposure to high concentrations of the gas, the hind legs remained paralyzed for an hour or more, the insects dragging themselves along by means of the front legs. Similarly, the foremost portion of the centipedes recovered first, the rear portion remaining paralyzed for an hour or more after removal from an exposure to 75 per cent butylene.

Carbon dioxide was a very effective anaesthetic for insects. Rose chafers were rendered insensible within 25 seconds with a mixture of 30 per cent carbon dioxide and 70 per cent oxygen. The animals recovered quickly after a 20-minute exposure with no apparent ill effects. Long exposures, however, caused paralysis of the legs somewhat as described for butylene.

Ethylene and acetylene, both of which will anaesthetize mammals if the concentration is near 80 per cent, were not effective on invertebrate animals until the concentration approached 100 per cent. In fact, they were no more effective than helium or nitrogen. This is in striking contrast with the effectiveness of these unsaturated hydrocarbons on plants.

The unsaturated hydrocarbons and carbon monoxide caused both epinasty of leaves and growth rigor resulting in retardation in elongation of stems. The former response due to stimulation, was reported in detail by Crocker, Zimmerman, and Hitchcock (2). Results showing the anaesthetic properties of the gases are shown in Table III. It is assumed that low concentrations of the gases caused partial anaesthesia bringing about a retardation in elongation of the stem. The percentage retardation increased with the concentration of the anaesthetic, finally reaching complete inhibition of growth. Sunflower and tomato plants were studied also by means of motion pictures while being exposed to 0.0005 per cent ethylene and compared with controls in normal atmosphere. The controls were in constant motion due to irregular growth on different parts of the stems and leaves, whereas the plants in ethylene developed pronounced epinasty of the leaves and then all movements ceased. Under these conditions the plants were considered to be completely anaesthetized.

Motion pictures were also made of *Mimosa pudica*, sensitive plant, while in 0.25 per cent carbon monoxide for 24 hours. Similar studies were reported by Zimmerman, Crocker, and Hitchcock (3) for *Mimosa pudica* exposed to 1 per cent carbon monoxide. In both cases the sensitive plants exposed to the carbon monoxide gas lost their sensitivity to external stimuli. The leaves assumed a ruffled appearance and moved about without correlation more rapidly than those of control plants. Neighboring leaves frequently became entangled and later separated with a jerk. The move-

ments of control leaves were orderly, and though the movement was up and down and from one side to the other, the range was comparatively narrow. A few hours after removal from the gas, the treated plants again resumed their normal equilibrium position to gravity and became sensitive to external stimuli. *Mimosa* responded similarly to the unsaturated hydrocarbons except butylene. In the latter case the plants withstood 10 per cent butylene with only slight loss in sensitivity to external stimuli and retaining the normal equilibrium position of the leaves. It is to be noted (Table III) also that 2 per cent butylene did not retard elongation of tobacco stems.

TABLE III

RETARDATION OF PLANT GROWTH FROM ANAESTHETICS

Name and No. of plants used in each experiment	Conc. of anaesthetic in per cent	Length of exposure in days	Av. elongation of stems during treatment in percentage
8 sweet pea plants	Control	3	105
	Ethylene 0.001	3	0
	Ethylene 0.0001	3	44
	Ethylene 0.00001	3	59
3 cucumber vines	Control	3	40
	Ethylene 0.001	3	12
	Ethylene 0.0001	3	8
	Ethylene 0.00001	3	19
4 heliotrope plants	Control	5	55
	Ethylene 0.001	5	12
	Acetylene 0.25	5	11
	Propylene 0.25	5	12
	Carbon monoxide 1.67	5	11
5 tobacco plants	Control	12	104
	Ethylene 0.001	12*	68
	Acetylene 0.1	12*	82
	Propylene 0.1	12*	64
	Carbon monoxide 1.67	12*	71
	Butylene 2.0	12*	137

* Plants were exposed two days then rested two days throughout the 12 day period.

DISCUSSION

At the outset the purpose of these investigations was to test the theory that carbon monoxide might properly be classified as an anaesthetic for plants, insects, and centipedes. No attempts have been made to consider the published theories on causes of anaesthesia, most of which are based on the results of experiments with animals involving ether or chloroform as the anaesthetics. Bernard (1, p. 255-267) proved in 1878 that the sensitive plant (*Mimosa pudica*) could be rendered insensible by means of ether and chloroform, his results serving to establish a close physiological relationship between the protoplasm of plants and animals.

One of the striking differences between plants and animals brought out in the present paper concerns the difference in concentrations of the chemical necessary to anaesthetize. Crocker, Zimmerman, and Hitchcock (2, p. 192–194) reported that one part of ethylene in 10,000,000 parts of air caused anaesthesia (growth rigor) in plants. This chemical is effective on mammals when air is replaced by 80 per cent ethylene mixed with 20 per cent oxygen. Insects and centipedes, however, were not affected until the concentration of ethylene approached 100 per cent, as was true also for nitrogen and helium. Plants were anaesthetized by 0.05 per cent of carbon monoxide, but 80 per cent or more was necessary to anaesthetize insects and centipedes. Comparative figures showing low concentrations of the different gases causing anaesthesia in plants and animals are shown in Table IV. The figures for plants in the table were based on concentrations of the gases which cause epinasty of leaves which response requires a higher dosage than that necessary to anaesthetize actively growing parts of the plant. Propylene and carbon monoxide were found to be very effective anaesthetics for insects and centipedes which were rendered insensible quickly and recovered quickly with no ill effects from long exposures to high concentrations. Of all the gases tested, butylene was the most toxic to animals and caused anaesthesia with the lowest concentration—5.0 per cent. The anaesthetic effect of this gas on plants is comparatively mild, 10 per cent causing *Mimosa* to become only slightly sluggish in response and 2 per cent causing no retardation in growth.

TABLE IV

COMPARATIVE EFFECTIVENESS OF ANAESTHETICS ON PLANTS AND ANIMALS

Anaesthetic	Lowest concentrations expressed in per cent of air	
	Causing anaesthesia in animals*	Causing epinasty in plants**
Ethylene	99.0 to 100.0†	0.00001
Acetylene	99.0 to 100.0†	0.0005
Propylene	30.0 to 75.0	0.0005
Carbon monoxide	80.0 to 85.0	0.05
Carbon dioxide	30.0	—
Butylene	5.0 to 40.0	—

* Data from Table II.
** Data from previous paper (2) on epinasty of leaves.
† Mammals are anaesthetized by 80 per cent or more.

Praying mantis, honeybee, bumblebee, yellow jacket, cockroach, and two species of grasshoppers were tested as described in detail for centipedes, rose chafer, and katydid. Considerable variation of the species in sensitivity to the various gases was discovered, but in general all would fall within the limits of the three that were described.

Except for *Mimosa pudica*, plants do not exhibit a good qualitative measure of anaesthesia. Other plants have been judged on the basis of retardation in growth (growth rigor) or decrease in growth movements. The degree of growth rigor varies with the concentration of the gas from slight retardation in elongation of the stem with low dosages to complete stoppage at higher concentrations.

As with animals, plant species showed considerable variation in their sensitivity to the anaesthetics. For example, tobacco plants in 0.001 per cent ethylene made approximately 68 per cent of normal growth while the growth of the sweet peas was stopped with that concentration of the gas.

SUMMARY

The anaesthetic effect of carbon monoxide, carbon dioxide, propylene, butylene, ethylene, and acetylene, when mixed with oxygen, was tested on ten different species of insects and centipedes. The lowest concentrations found to cause anaesthesia are given in per cent by volume as follows:

Propylene, for centipede, 30; katydid, 75; rose chafer, 60.
Carbon monoxide, for centipede, 81.5; katydid, 89, rose chafer, 85.
Butylene, for centipede, 5; katydid, 10; rose chafer, 40.
Ethylene or acetylene, for centipede, katydid, and rose chafer, 100.
Carbon dioxide, for rose chafer, 30.

In preliminary tests it was found that insects and centipedes remained apparently normal in nitrogen or helium mixtures with the oxygen content as low as 2 per cent.

Butylene was the most toxic of the anaesthetics tested. The animals exposed to this chemical became paralyzed if the concentration was much higher than that necessary to cause anaesthesia.

Ethylene and acetylene were the least effective of the anaesthetics for insects and centipedes, these gases being in a class with nitrogen and helium. This is in great contrast with their effectiveness on plants and mammals.

Ethylene was the most effective plant anaesthetic, 0.0005 per cent stopping growth movements of tomato and sunflower plants as shown by motion pictures; 0.001 per cent stopped elongation of sweet pea seedlings, while 0.00001 per cent retarded elongation nearly 50 per cent.

The degree of retardation in growth from ethylene gas varied with the concentration and the plant species.

Acetylene and propylene were about equally effective as plant anaesthetics. Both were approximately 10 times as effective as carbon monoxide.

Mimosa pudica lost its capacity to respond to external stimuli while being exposed to 0.25 per cent of carbon monoxide, but became normal again upon being removed from the gas.

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FURTHER EXPERIMENTS ON SHORTENING THE REST PERIOD OF POTATO TUBERS[1]

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The production of an autumn crop of Irish potatoes (*Solanum tuberosum* L.) is an essential feature of the agriculture of many of the southern and southwestern states of the United States and of Bermuda, Cuba, and Mexico. Since the tubers available for planting at this time of year often have been harvested for only a few days, or at most for a few weeks, previous to the time at which they must be planted, the slowness or irregularity of germination of such dormant or partially-dormant tubers has been a serious factor in the production of a successful crop at that time of year. And this problem affects not only the growers of the crops in these localities in which the autumn-planted crops are grown, but also those in the northern sections who furnish seed tubers from their summer-grown crop harvested in late summer or early autumn.

In previous reports (5, 6, 7) it was proposed that treatments of tubers with the thiocyanates of sodium, potassium, or ammonium, or with ethylene chlorhydrin ($ClCH_2CH_2OH$) might offer a solution of the problem of obtaining good germination of recently-harvested dormant tubers. Since that time tests of these suggested methods have been made by various workers in the United States, Canada, Bermuda (8), Czechoslovakia (19), and Russia (9). In some cases the reports have been favorable and in others unfavorable.

It is particularly with regard to the use of thiocyanate that unfavorable reports have come in. Rosa (18) found that it produced injury, and that non-toxic concentrations were ineffective in hastening sprouting. Cochran (3) found that although NH_4SCN broke the rest period the yield was lower than with untreated tubers because the treatment reduced the number of plants that became established. MacLeod (13) obtained good germination with NH_4SCN and $NaSCN$, but $KSCN$ produced only slight stimulation of sprouting. Because of these unfavorable reports experiments were undertaken to determine whether they could have been due to variation in concentration of chemical, in the stage of dormancy at the time of treatment, or in the temperature of the soil after planting. The results, described in detail in a later paragraph, show that even over a rather wide range of variation in these factors favorable effects upon sprouting were obtained. One of the objects of the present report is to emphasize the advantages of this very simple method of treating dormant potato tubers, and to urge that it is deserving of more extensive tests than have been given to it at present.

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In the more southerly portions of the United States it has been found that chemical treatments may not be necessary for forcing newly-harvested tubers and that merely storing the tubers at relatively high temperatures (about 30° to 35° C.) broke the rest period. The result, which is due to the experiments of Loomis (10) and of Rosa (17), was unexpected since it had been supposed previously that the most favorable storage conditions for shortening the rest period were at low rather than at high temperatures. But the time required for this storage period is about 30 days, and in the more northerly portions of the southern states this period of storage causes too much delay in planting. In the present report will be found results showing that chemical treatments can be used to shorten materially the length of this preliminary storage period.

Inquiries have been received regarding the possibility of treating freshly-harvested tubers in the North and shipping the treated tubers to the localities in the South in which they are to be planted. This plan involves the treatment of tubers in car-lot quantities in such a manner that the early stages of germination will take place during the time the cars are enroute. Small scale tests were made from this point of view and it was found that if the period of treatment with vapors of ethylene chlorhydrin was extended to about one week the treated tubers could be stored in air and that sprouts would develop in about 7 to 10 days. Experiments relating to the temperatures during the period of treatment and during the subsequent period of storage showed a fairly wide range within which treated tubers would give good germination when they were cut and planted subsequently in the soil. Preliminary measurements were made as to suitable concentrations of chemical, rate of absorption of vapors by the tissue, and amount of chemical retained by the tissue or decomposed by it. These data are expected to be of value in case there is a need of information on the problem of pre-treating tubers in large quantities for distance shipping.

RESULTS

EXPERIMENTS WITH SODIUM THIOCYANATE

The effect of various concentrations of sodium thiocyanate upon tubers in different stages of dormancy held at different temperatures after the cut tubers were planted in the soil is shown in Table I. The tubers were first cut into pieces weighing approximately one ounce each and were soaked for one hour in the solution of NaSCN using the concentrations shown in column 3. With the film of thiocyanate solution still clinging to the treated pieces they were planted in soil in flats which were placed in rooms regulated at the temperatures shown in column 2. Previous experiments had shown that the most favorable concentration of NaSCN was 1.0 per cent if the treated pieces were not rinsed with water after treatment.[2] The three

[2] In the early experiments (6, p. 387) the solution recommended was 2.0 per cent; but in that case the treated pieces were rinsed after treatment in order to remove the adhering chemical.

TABLE I

CONCENTRATION OF CHEMICAL, TEMPERATURE AFTER PLANTING, AND STAGE OF DORMANCY OF TUBERS AS FACTORS IN THE TREATMENT OF POTATOES WITH SODIUM THIOCYANATE

Var.	Air temp.	% NaSCN	% germination, days after planting				% rotten	Var.	Air temp.	% NaSCN	% germination, days after planting				% rotten
			20 days	30 days	40 days	60 days					20 days	30 days	40 days	60 days	
Irish Cobbler	Room	2.0	14	88	100		0	Bliss Triumph	Room	2.0	78	97	100		0
		1.0	44	95	100		0			1.0	84	96	100		0
		0.5	20	46	62	100	0			0.5	82	96	100		0
		Ck.	0	0	0	9	4			Ck.	33	58	83	100	0
	29° C.	2.0	0	69	86	96	4		29° C.	2.0	80	100			0
		1.0	67	100			0			1.0	93	100			0
		0.5	33	58	84	96	4			0.5	93	100			0
		Ck.	0	0	0	23	0			Ck.	13	40	67	96	0
	35° C.	2.0	0	45	100		0		35° C.	2.0	20	84	100		0
		1.0	59	91	100		0			1.0	48	94	100		0
		0.5	52	91	100		0			0.5	66	92	96		4
		Ck.	12	33	56	89	4			Ck.	33	78	80		20
Irish Cobbler	Room	2.0	8	10			90	Green Mountain	Room	2.0	87	100			0
		1.0	42	62	76	96	4			1.0	99	100			0
		0.5	10	45	61	96	4			0.5	100				0
		Ck.	0	0	0	0	20			Ck.	85	100			0
	29° C.	2.0	24	53	64	64	36		29° C.	2.0	70	100			0
		1.0	24	62	82	96	4			1.0	98	100			0
		0.5	7	51	77	89	4			0.5	95	100			0
		Ck.	0	0	0	16	16			Ck.	97	100			0
	35° C.	2.0	0	46	70	76	24		35° C.	2.0	21	64	76		24
		1.0	0	44	100		0			1.0	71	86	92		8
		0.5	0	58	90	100	0			0.5	79	100			0
		Ck.	0	0	44	100	0			Ck.	87	100			0
Irish Cobbler	Room	2.0	30	72	100		0	Bliss Triumph	Room	2.0	80	96			4
		1.0	91	100			0			1.0	100				0
		0.5	68	88	95	100	0			0.5	100				0
		Ck.	0	12	19	27	0			Ck.	100				0
	29° C.	2.0	24	92	100		0		29° C.	2.0	100				0
		1.0	56	96	100		0			1.0	100				0
		0.5	67	88	92	96	4			0.5	100				0
		Ck.	0	8	18	71	0			Ck.	100				0
	35° C.	2.0	14	84	96		4		35° C.	2.0	28	76	84		16
		1.0	43	100			0			1.0	83	100			0
		0.5	59	84	100		0			0.5	92	100			0
		Ck.	8	40	46	72	28			Ck.	88	100			0
Bliss Triumph	Room	2.0	44	100			0	Irish Cobbler	Room	2.0	53	100			0
		1.0	100				0			1.0	100				0
		0.5	80	96	100		0			0.5	100				0
		Ck.	0	0	58	96	0			Ck.	100				0
	29° C.	2.0	68	96			4		29° C.	2.0	100				0
		1.0	96				4			1.0	100				0
		0.5	60	92	100		0			0.5	100				0
		Ck.	0	32	98	100	0			Ck.	100				0
	35° C.	2.0	16	48	88		12		35° C.	2.0	36	79	92		8
		1.0	28	83	100		0			1.0	78	100			0
		0.5	48	80	96		4			0.5	100				0
		Ck.	20	57	98	100	0			Ck.	100				0

concentrations chosen for this experiment, therefore, represent the optimum, twice the optimum, and one-half the optimum. The temperatures indicated in column 2 are the air temperatures of the rooms in which the flats of planted potatoes were stored. The soil temperatures were somewhat lower than this due to the cooling effect of evaporation from the moist soil. Thermometers inserted into the planted potatoes showed temperatures of about 20° C. in the room temperature lot, 25° C. in the 29° room, and 30° C. in the 35° room. The flats were examined each 4 to 7 days and the seed pieces which had produced visible sprouts were removed after a record had been made of the germination. The rate of emergence of sprouts in the different lots is shown in columns 4 to 7, and the percentage of rotten seed pieces in column 8.

The tubers used in the experiments described in Table I were chosen to represent various stages of dormancy and have been arranged in the table approximately in the order of dormancy. The Irish Cobbler lot at the top on the left hand half of Table I was very dormant while the lot of tubers at the bottom of the right hand half of Table I was not at all dormant but had well-developed sprouts at the time the chemical treatments were applied.

Table I shows that favorable results were obtained with 1.0 per cent NaSCN at all temperatures and with potatoes in all stages of dormancy. Rapid sprouting of dormant potatoes was obtained and no injury resulted from treating potatoes that were not dormant. When the concentration of NaSCN was reduced to 0.5 per cent the gain in the rate of germination of the dormant tubers was only slightly less than with 1.0 per cent. However, when the concentration was increased to 2.0 per cent distinctly unfavorable results were obtained in many cases, there being a greater percentage of rotten seed pieces, and, with the non-dormant lots of tubers, a distinct delay in germination. There was some tendency for a greater percentage of rot in the check lots than in those treated with either 1.0 or 0.5 per cent NaSCN, a result which was due probably to the greater length of time the seed pieces of the check lots were required to remain in the soil because of slowness of germination.

COMBINATIONS OF STORAGE TEMPERATURES AND CHEMICAL TREATMENTS

Because of the favorable results obtained by Loomis (10), Rosa (17), and Cooper (4) in storing dormant tubers at relatively high temperatures, experiments were undertaken to determine whether a chemical treatment could be applied with advantage to tubers which had been stored at high temperatures, and whether this treatment would shorten the time required for the storage period.

Loomis recommended temperatures of 30° to 33° C. (10, p. 292) and 35° C. (11). In the present experiments the data in Table I on the germination of the untreated tubers at different temperatures show much more favorable germination at 35° than at 29°, and, although these results were obtained with cut tubers planted in the soil, other experiments not reported here showed that in the storage of whole tubers in air 35° was much superior to 29° for hastening germination. Consequently 35° was selected as the storage temperature for these tests, and in order to avoid too long storage at this temperature samples were removed at intervals after the start of the storage.

The tubers were stored in paper bags in a room maintained at 35°. A thermometer inserted into certain of the tubers showed that the tubers attained this temperature very closely. At intervals of 10, 18, and 30 days of storage, samples of tubers were removed, cut into pieces, and either planted without treatment, or after treatment with chemicals. The sodium thiocyanate treatment consisted in soaking the cut tubers in 1.0 per cent NaSCN for one hour and planting the treated pieces without rinsing. For the ethylene chlorhydrin treatment the cut tubers were dipped momentarily in a solution prepared by dissolving 50 cc. of 40 per cent ethylene chlorhydrin in a liter of water, after which they were placed at once in Mason fruit jars for 24 hours at 22° C.

In order to determine whether the stage of the rest period at which the high temperature-storage began was a factor, the storage tests were started at intervals of 7, 14, and 28 days after harvest.

The results for Bliss Trimph are shown in Table II and for Irish Cobbler in Table III. In the experiments started either 7 or 14 days after harvest, gains of 20 to 40 days, or even more, in the time required for sprouting were obtained by treating the tubers with chemicals, even though they had been stored at the high temperature for periods of 10 or 18 days before the treatment. A storage period of 30 days at 35° was injurious to the Bliss Triumph tubers. The Irish Cobbler endured 30 days storage at 35°, but even after this period of temperature-storage, chemical treatments hastened the germination of the samples of tubers placed in storage at this more dormant stage.

In the experiments started 28 days after harvest the chemical treatments showed gains over the corresponding lots stored at 35° for either 10 or 18 days, these gains being more than 30 days for the Irish Cobbler tubers, but only about 10 days for Bliss Triumph.

Gains in sprouting due to temperature-storage alone without chemical treatment were not striking especially when allowance is made for the time required for the completion of the storage period before planting. With Bliss Triumph it is only with the experiment started 28 days after harvest that gains by temperature-storage were greater than the time required for

TABLE II

EFFECT OF STORAGE TEMPERATURE WITH AND WITHOUT CHEMICAL TREATMENT. BLISS TRIUMPH VARIETY

Days after harvest before experiment started	Days storage at 35° C. before treatment	Chemical treatment after storage	% germination, days after planting					% rotten
			20 days	30 days	40 days	50 days	60 days	
7	0	NaSCN	77	90	94	96		4
		C_2H_4ClOH	89	92	94			6
		None	0	30	52	75	84	2
	10	NaSCN	92	100				0
		C_2H_4ClOH	82	86	90			10
		None	0	28	42	62	75	0
	18	NaSCN	96	100				0
		C_2H_4ClOH	84	98				2
		None	0	61	85	94	96	2
	30	NaSCN	30	49				51
		C_2H_4ClOH	52	63				37
		None	44	74	76			24
14	0	NaSCN	86	100				0
		C_2H_4ClOH	81	96				4
		None	0	15	21	34	48	4
	10	NaSCN	40	95	100			0
		C_2H_4ClOH	86	100				0
		None	8	20	40	57	73	4
	18	NaSCN	92	98				2
		C_2H_4ClOH	89	96				4
		None	11	34	59	80	100	0
	30	NaSCN	68	76				24
		C_2H_4ClOH	74	80				20
		None	74	84				16
28	0	NaSCN	95	98				2
		C_2H_4ClOH	22	75	96			4
		None	0	12	30	37	45	0
	10	NaSCN	100					0
		C_2H_4ClOH	98					2
		None	68	96	100			0
	18	NaSCN	80	96				4
		C_2H_4ClOH	94					6
		None	66	84	94			6
	30	NaSCN	8					42
		C_2H_4ClOH	18					82
		None	14					86

the storage period. With Irish Cobbler the gains were in most cases greater than the time needed for the storage period itself but a 30-day period of storage was needed for good germination of tubers at the 7- and 14-day

TABLE III

EFFECT OF STORAGE TEMPERATURE WITH AND WITHOUT CHEMICAL TREATMENT. IRISH COBBLER VARIETY

Days after harvest before experiment started	Days storage at 35° C. before treatment	Chemical treatment after storage	% germination, days after planting					% rotten
			20 days	30 days	40 days	50 days	60 days	
7	0	NaSCN	56	79	84	94	100	0
		C_2H_4ClOH	66	76	84	90		10
		None	0	0	0	6	10	0
	10	NaSCN	93	100				0
		C_2H_4ClOH	77	92	100			0
		None	0	14	24	39	45	0
	18	NaSCN	91	98				2
		C_2H_4ClOH	34	61	80	90	98	2
		None	0	12	28	46	60	0
	30	NaSCN	49	94	98			2
		C_2H_4ClOH	44	79	90	92		8
		None	0	23	42	64	85	10
14	0	NaSCN	70	100				0
		C_2H_4ClOH	79	89	90	92	94	6
		None	0	0	0	0	0	0
	10	NaSCN	54	98				2
		C_2H_4ClOH	40	60	79	95	96	4
		None	0	0	0	0	6	0
	18	NaSCN	82	100				0
		C_2H_4ClOH	42	76	96	100		0
		None	0	0	0	22	56	0
	30	NaSCN	72	92	96			4
		C_2H_4ClOH	85	92	96			4
		None	45	83	96			4
28	0	NaSCN	87	97	100			0
		C_2H_4ClOH	65	85	96	100		0
		None	0	0	0	4	24	0
	10	NaSCN	90	100				0
		C_2H_4ClOH	100					0
		None	0	0	20	38	58	0
	18	NaSCN	75	100				0
		C_2H_4ClOH	65	94	96			4
		None	0	15	31	52	76	0
	30	NaSCN	36					64
		C_2H_4ClOH	90					10
		None	82					18

periods after harvest and in these cases treatment with chemicals induced still further gains in germination.

Furthermore, the results show that the margin of time for storage at

35° C. at which the rest period of the tubers could be markedly shortened without at the same time causing injury to them was rather narrow, 18 days being too short a time for all lots except possibly the least dormant stage of Bliss Triumph, and 30 days being too long a time for all lots except the most dormant stage of Irish Cobbler.

The results in general indicate that even after a period of storage of tubers at 35° C. a gain in germination can be obtained by treating the stored tubers with either NaSCN or ethylene chlorhydrin. This is especially true of tubers that had been harvested for only a short time when placed in storage at the high temperature.

PRE-TREATMENT OF WHOLE TUBERS FOR DISTANCE SHIPPING

The object of the experiment was to determine whether it is likely that potatoes shortly after harvest in the North could be given a chemical treatment which would break their dormancy and allow them to be shipped to the South and used successfully for the planting of an autumn crop in that same year.

Whole tubers which had been harvested 7 to 10 days previously were exposed to vapors of ethylene chlorhydrin in a closed container for a period of 7 days, after which the treated tubers were stored in paper bags for 14 days, a period intended to be comparable with the time required for shipment from the northern to the southern portions of the United States.

The chemical used was 40 per cent ethylene chlorhydrin and the amounts shown in column 3, Table IV for each variety were placed in cheesecloths which were spread loosely on shelves in the upper portions of the containers. Evaporation occurred spontaneously without the aid of stirrers, the use of which was impracticable because of the large number of tests which were carried out simultaneously.

At the end of the treatment samples of tubers were placed in paper bags and were stored in rooms maintained at the temperatures shown in column 2 for each variety. After storage in these rooms for 14 days the tubers were cut into approximately 1-ounce pieces and were planted in soil in flats.

The data in table IV show the per cent germination in the different lots at intervals of 20, 30, 40, 60, and 90 days after planting. With Bliss Triumph successful sprouting without rotting of seed pieces was obtained with 5 cc. of chemical per 30 l. of space within the container. Higher concentrations were favorable with the treatments at 22° C., but at 29° C. the higher amounts caused injury. With Irish Cobbler better results were obtained with 9 cc. than with 5 cc. but both concentrations hastened germination by at least 30 to 60 days without any serious injury to seed pieces whether the temperature of treatment was 22° or 29° C.

Soil temperature may be a factor in the success of a method of pre-

TABLE IV

TREATMENT OF WHOLE TUBERS WITH ETHYLENE CHLORHYDRIN. EFFECT OF TEMPERATURE DURING TREATMENT AND SUBSEQUENT STORAGE

Variety, Bliss Triumph

Temp. of treat-ment	Temp. of storage	Cc. of chemical in 30 l. space	% germination, days after planting: 20 days	30 days	40 days	60 days	90 days	% rotten
22° C.	15° C.	15	96	100				0
		9	96	100				0
		5	94	100				0
		Check	0	30	42	66	98	0
	22° C.	15	79	84	86			14
		9	84	88	89			11
		5	90	96	100			0
		Check	0	28	50	88	100	0
	29° C.	15	56	64				36
		9	88	96				0
		5	74	87	93	96		0
		Check	13	28	41	68	100	0
29° C.	15° C.	15						100
		9						100
		5	91	96	96	98		0
		Check	0	30	51	64	95	0
	22° C.	15						100
		9						100
		5	80	88	91	100		0
		Check	16	44	86	94	100	0
	29° C.	15						100
		9						100
		5	68	86	92			8
		Check	0	38	55	84	97	0

Variety, Irish Cobbler

Temp. of treat-ment	Temp. of storage	Cc. of chemical in 30 l. space	% germination, days after planting: 20 days	30 days	40 days	60 days	90 days	% rotten
22° C.	15° C.	15	94	100				0
		9	50	64	77	100		0
		5	32	52	61	82	86	0
		Check	0	0	0	0	25	0
	22° C.	15	84	95	100			0
		9	73	85	92	100		0
		5	60	76	86	100		0
		Check	0	0	0	20	35	0
	29° C.	15	76	82	89	96		4
		9	53	64	71	100		0
		5	33	47	61	84	100	0
		Check	5	9	11	29	43	0
29° C.	15° C.	15						100
		9	74	100				0
		5	38	60	76	100		0
		Check	0	16	24	58	77	4
	22° C.	15						100
		9	87	96	98			2
		5	71	84	92	100		0
		Check	0	17	28	50	67	0
	29° C.	15						100
		9	77	89	98			2
		5	45	65	80	94	98	2
		Check	0	13	24	50	68	0

treatment of tubers and the results of a test made on this phase of the problem are shown in Table V. The tubers were first treated at 22° for 7 days, then stored 14 days at 22°, and finally were planted in soil in flats which were placed in constant temperature rooms at the temperatures of 15° and 29° C. shown in column 2, Table V. The results with concentrations of either 5 or 9 cc. of 40 per cent ethylene chlorhydrin in the 30-l. containers were favorable with both varieties at both temperatures.

TABLE V

EFFECT OF TEMPERATURE AFTER PLANTING

Variety	Air temp. after planting	Cc. of C_2H_4ClOH in 30 l. space	% germination, days after planting					% rotten
			20 days	30 days	40 days	60 days	90 days	
Bliss Triumph	15° C.	15	95	100				0
		9	77	90	95	96	98	2
		5	56	69	75	100		0
		Check	0	0	0	48	85	0
	29° C.	15	56	64	76			24
		9	94					6
		5	78	92	96			4
		Check	30	72	91	94		6
Irish Cobbler	15° C.	15	71	82	86	100		0
		9	90	97	100			0
		5	54	66	74	100		0
		Check	0	0	0	0	84	0
	29° C.	15	79	100				0
		9	79	100				0
		5	51	70	86	100		0
		Check	14	30	47	66	88	12

Tests were made with Maine-grown tubers[3] shipped by express from Maine to Yonkers arriving about 10 days after they had been harvested. The results are shown in Table VI. In this experiment smaller containers were used and the amount of chemical was adjusted to give a somewhat lower range of concentrations than that used in the experiments previously described. The rate of germination was markedly hastened and the only difficulty encountered was in the tests at 29°. Lots treated for 7 days at this temperature germinated well if the tubers were stored at 15° or 22° before planting, but if stored at 29° injury was obtained with the lots receiving 3.5 cc. of chemical per 17.5-l. container. Some preliminary tests indicate that this injury at continuously high temperatures during treatment can be avoided by shortening the period of treatment under such conditions to about 4 days.

[3] The authors express thanks to Dr. E. S. Schultz of the United States Department of Agriculture for furnishing these supplies of experimental tubers.

TABLE VI
RESULTS WITH MAINE-GROWN TUBERS

Variety	Temp. of treatment	Temp. of storage	Cc. of C_2H_4ClOH in 17.5 l. space	% germination, days after planting: 20 days	30 days	40 days	60 days	90 days	% rotten
Bliss Triumph	22° C.	15° C.	3.50	94	100				0
			1.75	48	65	73	93	100	0
			Check	0	0	25	64	100	0
		22° C.	3.50	100					0
			1.75	70	83	91	100		0
			Check	0	0	28	70	100	0
		29° C.	3.50	100					0
			1.75	67	82	87	100		0
			Check	28	47	58	95	100	0
	29° C.	15° C.	3.50	100					0
			1.75	83	100				0
			Check	0	19	39	75	100	0
		22° C.	3.50	100					0
			1.75	83	92	100			0
			Check	0	41	61	92	100	0
		29° C.	3.50						100
			1.75	98					2
			Check	0	36	63	83	100	0
Irish Cobbler	22° C.	15° C.	3.50	40	52	60	83	100	0
			1.75	11	19	32	60	100	0
			Check	0	0	0	0	64	0
		22° C.	3.50	62	82	86	100		0
			1.75	27	39	58	85	100	0
			Check	0	0	0	21	70	0
		29° C.	3.50						100
			1.75	29	39	51	79	100	0
			Check	0	0	0	20	74	0
	29° C.	15° C.	3.50	62	76	88	100		0
			1.75	18	30	37	69	98	0
			Check	0	0	0	20	66	0
		22° C.	3.50	66	78	84	96	100	0
			1.75	25	42	58	86	100	0
			Check	0	0	0	20	72	0
		29° C.	3.50						100
			1.75	28	44	52	81	100	0
			Check	0	0	0	10	80	0

EFFECT OF TIME OF STORAGE AFTER TREATING BEFORE PLANTING

It had been found previously (6, p. 392) that when whole tubers are treated with vapors of ethylene chlorhydrin, at least with the concentra-

tions used, it is undesirable to cut the tubers into pieces and plant them at once, but that they should be stored in air and planted at a later period.

This point was tested again in the present experiments and a similar result was obtained. The results of this test with Maine-grown Bliss Triumph tubers are shown in Table VII. After the tubers had been treated for 7 days, using 5 cc. of 40 per cent ethylene chlorhydrin in 17.5-l. containers, the treated tubers were divided into four lots, one lot being cut into pieces and planted at once, the others being stored in paper bags in air for 3, 7, and 14 days, and then cut into pieces and planted. The germination record shown in Table VII is based on the number of days from the end of the treatment. It is seen that the lot planted at once after treatment showed 28 per cent rotten seed pieces, but that the lots stored in air after treatment germinated promptly when planted and showed no rot, at least in the plantings made on the 7th and 14th days after treatment. Probably the optimum time for planting to get early germination and to avoid rotting is about 5 to 7 days after treatment. In fact, not much time is lost by storing for 14 days as is shown by the good agreement in percentage germination on the 25th day after treatment of the lots stored 3, 7, and 14 days. It appears that the processes leading to sprout development take place nearly as well in the whole tuber stored in air as in the cut pieces planted in soil.

TABLE VII

EFFECT OF A STORAGE PERIOD AFTER TREATING BEFORE PLANTING

Chemical treatment	Days after treatment before planting	% germination, days after end of treatment							% rotten
		20 days	25 days	30 days	40 days	50 days	60 days	70 days	
With ethylene chlorhydrin	0	39	60	72					28
	3	74	90	96					4
	7	59	84	100					0
	14	0	84	100					0
Not treated	0	0	0	0	23	45	90	100	0
	3	0	0	0	25	54	92	100	0
	7	0	0	0	0	45	90	96	4
	14	0	0	0	30	75	97	100	0

DETERMINATION OF THE VAPOR PRESSURE OF ETHYLENE CHLORHYDRIN

Since, in the methods of treatment considered in this paper, the chlorhydrin can reach the tubers only as vapor mixed with the air surrounding them, it is desirable to know the concentration of vapor which can be obtained at various temperatures, the effectiveness with which the chlorhydrin evaporates into the air from the cheesecloth to which it is applied for the treatments, and the amount of chlorhydrin taken up by the tubers

during the course of the exposure. The maximum amount of chlorhydrin which can exist as vapor at a given temperature is determined by the partial pressure due to the chlorhydrin in the solution with which it is in equilibrium. Once the saturation point is reached the presence of an additional amount of the liquid phase cannot cause any further increase in the concentration of the vapor in the air. Of course, if the potatoes take up an appreciable amount of the vapor or if there is any loss of chlorhydrin through leakage or from any other source, an excess of liquid chlorhydrin is necessary to replenish the chlorhydrin thus removed from the vapor phase. The presence of an amount of chlorhydrin over and above that theoretically required to saturate the given air space may thus be desirable or even necessary for the most effective treatment.

These factors have been studied and the results show that the amount of chlorhydrin which has been found most efficacious is above the theoretical quantity necessary to saturate the air space available, and that in fact the tubers take up considerable quantities of chlorhydrin, which correspond to several times the maximum content of the air in the space in the closed containers used for the treatments.

As far as the authors are aware there are no published data giving the vapor pressure of either pure chlorhydrin or of the constant boiling mixture at room temperature. Bozza and Gallarati (2) have determined the boiling points at 760, 504, and 400 mm. pressure but give no figures for lower pressures.

The vapor pressure curves for both pure ethylene chlorhydrin and for the azeotropic mixture were determined by finding the boiling points at various pressures. The apparatus and procedure described by Mack and France (12, p. 47) were used. The chlorhydrin for the determinations was obtained from commercial products which were purified by several redistillations, the first and last fractions being discarded in each case. The 100 per cent chlorhydrin thus obtained boiled at 126.8° to 127.0°C. (uncorr.) at 756 mm. pressure and the constant boiling mixture at 97.6°C. (corr.) at 746 mm. The thermometers used for the vapor pressure determination were calibrated by comparison with Bureau of Standards thermometers and the temperature readings were corrected for stem emergence.

The data obtained are plotted in Figure 1. The boiling points at the three pressures studied by Bozza and Gallarati (2) are also shown. They are represented by crosses. In order to be able more readily to read off the vapor pressure at definite temperatures it is desirable to plot the data in such a form as to give straight lines. This can be done by plotting the reciprocal of the absolute temperature of the boiling point at a given pressure against the reciprocal of the absolute temperature of the boiling point of water at that pressure [White (23)]. The lines thus obtained are shown in Figure 2. The points corresponding to the data of Bozza and

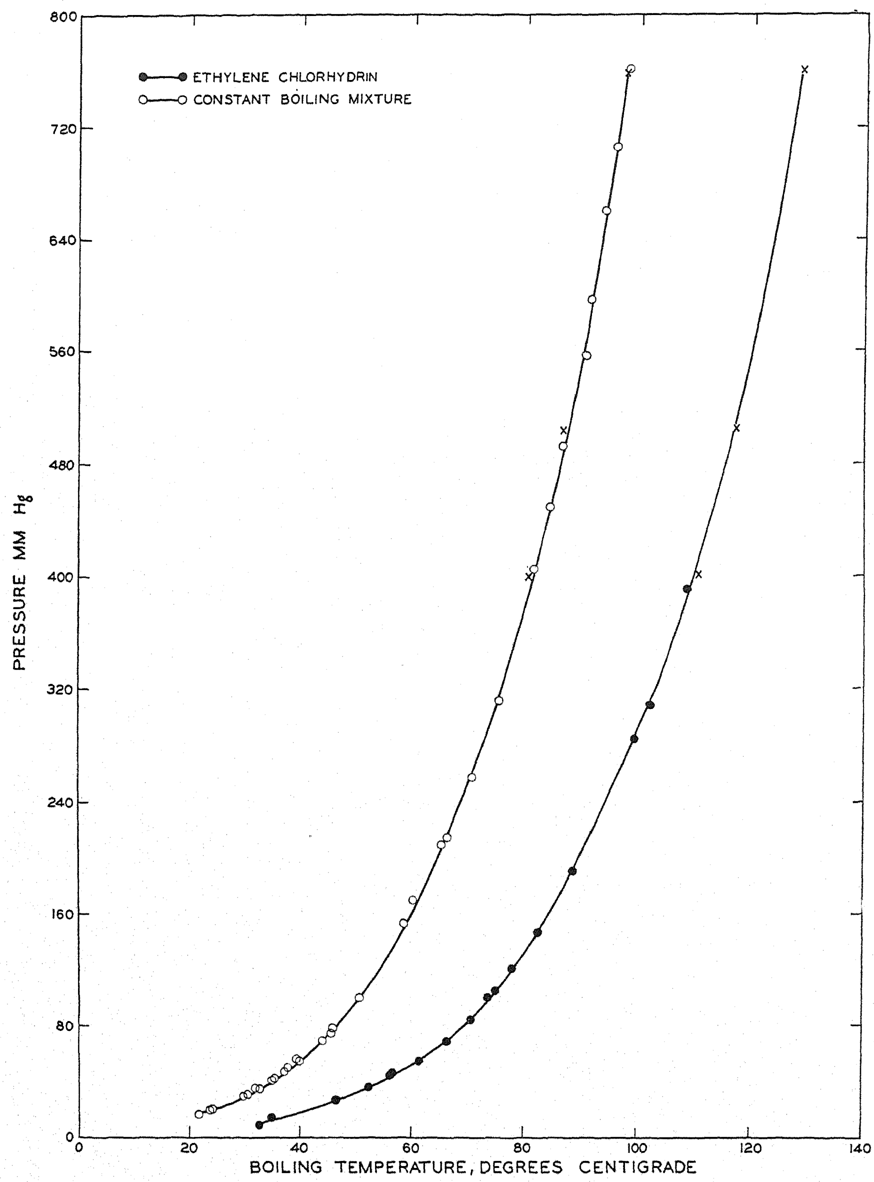

FIGURE 1. The vapor pressure of ethylene chlorhydrin and of its constant boiling mixture with water.

Gallarati are again shown by crosses. The data for the vapor pressure of water were obtained from the tables given in Van Nostrand's Chemical Annual (22, p. 726).

In order to determine the concentration of ethylene chlorhydrin vapor in equilibrium with the constant boiling mixture at various temperatures it is also necessary to know the percentage of chlorhydrin in the vapor above the constant boiling mixture at various temperatures. The percentage composition of the vapor above the constant boiling mixture at the boiling point at atmospheric pressure is known but this is not necessarily the same at lower pressures. Bozza and Gallarati report that the

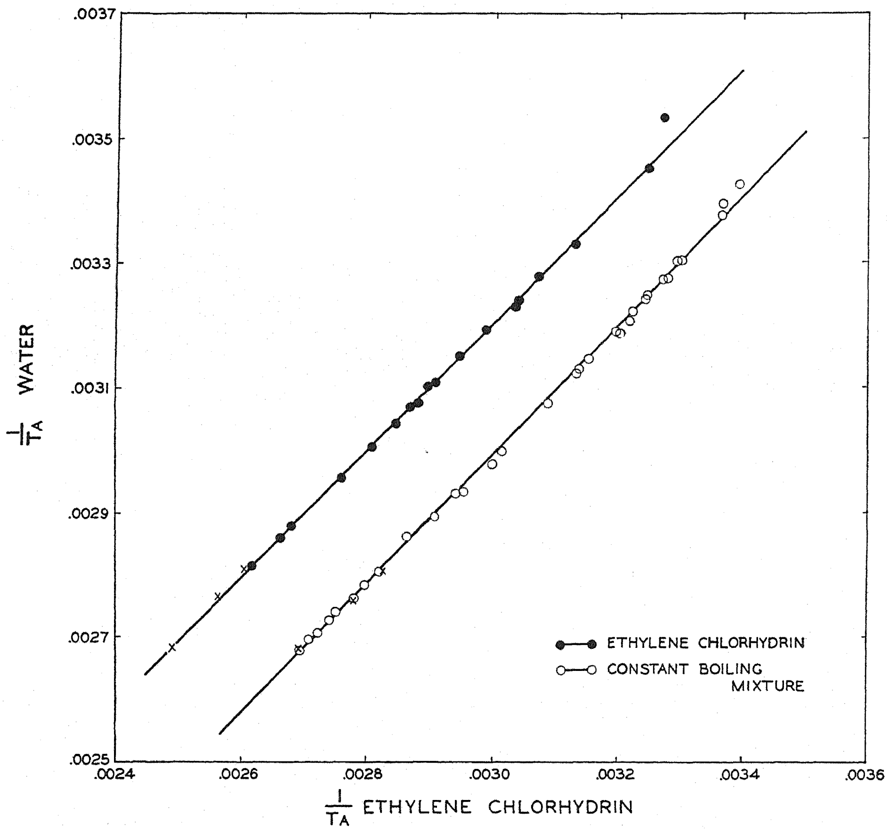

FIGURE 2. Plot of reciprocal temperatures at equal vapor pressures.

aqueous mixture of minimum boiling point at 400 mm. contains 40.5 per cent of chlorhydrin. No values are given for lower pressures.

The composition of the vapor above the constant boiling mixture at various pressures was determined by distilling over small portions from a relatively large volume and determining their density. Specific gravity bottles of 2 cc. capacity were used and duplicate portions were obtained at each temperature studied. For the density determinations the temperature was adjusted to 25° C. by means of a thermostatically controlled water

bath, the temperature of which varied only about 0.05° on either side. By this method it was possible to determine the density with accuracy to the fourth decimal place. Good agreement was obtained between duplicates. It was found that the density of the portions collected at pressures of 11 to 18 mm. of mercury, corresponding to temperatures of 22° C. to 24° C., averaged 0.0052 gram lower than the density of the solution started with. According to the data of Radulescu and Muresanu (16) who showed that the density varies in a linear manner with concentration, this difference in density corresponds to a difference of 2.2 per cent in the amount of chlorhydrin present. It was thus found that the composition of the vapor over the constant boiling mixture does not change much with temperature.

From the data now at hand it was possible to determine how much ethylene chlorhydrin is present in a saturated atmosphere at a given temperature with either pure chlorhydrin or the constant boiling mixture as the saturating agent. These data for temperatures from 10° C. to 50° C. at 5° intervals are given in Table VIII. At 25° C., for example, to completely saturate one liter of air space 0.031 cc. of the constant boiling mixture is required. This corresponds to 0.0134 gram of pure ethylene chlorhydrin. If 100 per cent chlorhydrin is used 0.028 cc. or 0.0338 gram is required to saturate one liter at 25°.

TABLE VIII

VAPOR PRESSURE OF ETHYLENE CHLORHYDRIN AND OF ITS CONSTANT BOILING MIXTURE WITH WATER AT VARIOUS TEMPERATURES, AND QUANTITIES REQUIRED TO SATURATE ONE LITER OF AIR SPACE

Temp. ° C.	Ethylene chlorhydrin			Constant boiling ethylene chlorhydrin			
	Vapor pressure	Amt. required to saturate 1 liter		Vapor pressure	Partial pressure due to chlorhydrin*	Amt. required to saturate 1 liter	
		Grams	Cc.			Grams chlorhydrin	Cc. constant boiling
10	2.9	0.0132	0.011	8.8	1.2	0.00547	0.013
15	4.2	0.0188	0.016	12.3	1.6	0.00717	0.016
20	5.7	0.0251	0.021	17.2	2.3	0.0101	0.023
25	7.8	0.0338	0.028	23.5	3.1	0.0134	0.031
30	10.5	0.0447	0.037	31.6	4.2	0.0179	0.041
35	14.3	0.0599	0.050	42.3	5.6	0.0235	0.054
40	18.5	0.0763	0.064	55.0	7.3	0.0301	0.069
45	24.3	0.0987	0.082	69.7	9.2	0.0373	0.085
50	31.6	0.1263	0.105	98.0	12.9	0.0524	0.120

* Calculated on the basis of 40.5% chlorhydrin in vapor.

QUANTITY OF ETHYLENE CHLORHYDRIN TAKEN UP BY THE TUBERS DURING TREATMENT

To determine the amount of ethylene chlorhydrin taken up by the tubers during the exposure to the vapor it is necessary to have a method

which is suitable for the determination of relatively small quantities of chlorhydrin. The methods most commonly used for the quantitative estimation of chlorhydrin, the determination of the density or index of refraction, which vary in a linear manner with concentration (16), are not readily applicable to small quantities. In connection with the work on the determination of the CO_2 output of potatoes which had been treated with ethylene chlorhydrin it was observed that when conditions were such that some chlorhydrin vapor passed through the $Ba(OH)_2$ absorption tubes, chloride was present in the first tube in the chain and in the first one only. This indicated that the $Ba(OH)_2$ reacted with the ethylene chlorhydrin and that under the conditions prevailing all of the chlorhydrin was acted upon in the first tube. If this were not the case some chloride could have been detected in the second tube since ethylene chlorhydrin forms a constant boiling mixture of minimum boiling point and unaltered chlorhydrin would have passed into the second tube and formed $BaCl_2$ there.

Experiments showed that this reaction could be adapted for the quantitative determination of ethylene chlorhydrin. It is necessary to allow the $Ba(OH)_2$ and chlorhydrin mixture to stand for some time to permit completion of the decomposition of the chlorhydrin. The kinetics of the reaction between alkaline substances and chlorhydrin has been studied by Smith (20). The larger the quantity of $Ba(OH)_2$ present in comparison to the amount of chlorhydrin the more rapid the reaction. In the determinations carried out in the experiments reported in this paper the mixtures were allowed to stand at least 18 hours. The chloride was determined by adding an excess of N/20 $AgNO_3$ solution in the presence of nitric acid and titrating back with N/20 KSCN using ferric alum as an indicator.

In the earlier experiments the amount of ethylene chlorhydrin absorbed by the tubers was determined by difference. Duplicate desiccators were set up in each of which was placed a weighed amount of pure chlorhydrin. One desiccator contained 750 grams of tubers while the other was carried along as a blank containing only the added chlorhydrin. The desiccators were left undisturbed for 24 hours (27 hours in one case) after which a stream of air was drawn through the system in such a way that the gases leaving the desiccators passed through $Ba(OH)_2$ solutions. At suitable intervals the amount of chloride in the $Ba(OH)_2$ was determined. The difference between the amount recovered from the blank desiccator and from the desiccator with the tubers was considered as the amount taken up and retained by the tubers. The data are shown in Tables IX and X.

It is seen that in the case of the blank desiccators 97.0 and 97.8 per cent of the added chlorhydrin was recovered, thus indicating that the methods employed were satisfactory. It was found that 27.8 and 36.6 mg. of ethylene chlorhydrin per 100 grams of tubers were retained by the

TABLE IX

DETERMINATION OF ETHYLENE CHLORHYDRIN ABSORBED BY TUBERS

Hours from start of treatment	Chlorhydrin recovered from container with tubers (750 g.), cc. N/10	Chlorhydrin recovered from blank container, cc. N/10
27– 29	6.37	11.37
29– 48	22.70	39.06
48– 72	27.00	36.30
72– 96	15.75	22.17
96–144	4.87	3.50
Retained on cotton	1.20	0.37
Totals	77.89	112.77
Amount applied, cc. N/10	114.5	116.6
Per cent recovered	67.8	97.8
Amt. retained by tubers, mg. per 100 grams	36.6	—

tubers. Dormant tubers of the Irish Cobbler variety obtained from two different sources were used for the two experiments.

In one experiment two 750-gram portions of tubers were treated so that in one case the potatoes could be removed from the desiccators after the treatment period, washed, and placed into a clean desiccator and the chlorhydrin vapor given off by the tubers after treatment determined. That tubers give off some unaltered chlorhydrin after treatment had been observed in experiments on the effect of these treatments on the CO_2 output (15, p. 12). In this test (column 3, Table X) 9.6 mg. per 100 grams of tubers were given off in the first 48 hours after the end of treatment.

TABLE X

DETERMINATION OF ETHYLENE CHLORHYDRIN ABSORBED BY TUBERS

Hours from start of treatment	Chlorhydrin recovered from container with tubers (750 g.), cc. N/10	Chlorhydrin recovered unaltered from treated tubers only (750 g.), cc. N/10	Chlorhydrin recovered from blank container, cc. N/10
24.0– 43.5	34.75	8.85	35.2
43.5– 70.33	25.15	0.12	40.4
70.3– 96.5	18.60	Trace	22.6
96.5–166.8	5.00	Slight trace	10.6
166.8–238.5	Trace	None	0.35
Totals	83.50	8.97	109.15
Amt. applied, cc. N/10	112.7	114.5	112.8
Per cent recovered	74.1	7.8	97.0
Amt. retained by tubers, mg. per 100 grams	27.8	—	—

These results show that relatively large quantities of ethylene chlorhydrin are taken up by the tubers during the treatment period. Amounts of this magnitude can be determined directly by recovering by distillation the chlorhydrin contained in the tubers. The procedure adopted is as follows: Samples of 300 grams are cut into pieces weighing about two grams each and placed into two-liter Florence flasks together with 400 cc. of water. Three hundred cc. are distilled over into volumetric flasks containing 4.5 grams of solid $Ba(OH)_2$ per 100 cc. The potato mixture is then allowed to cool and an additional 100 cc. of water are added and another 100 cc. distilled over. A Kjeldahl-connecting bulb is inserted between the flasks and the condenser. It is necessary to conduct the distillation carefully both to prevent excessive foaming and to prevent charring of the pieces of potato tuber and subsequent breaking of the flasks. The flasks containing the $Ba(OH)_2$ and the distillate were titrated for their chloride content as indicated above after standing at least 18 hours.

Such a method gives somewhat low results. Losses are due chiefly to two causes. First, some chlorhydrin will be left in the mixture in the flasks after 400 cc. of distillate have been collected, and second, a certain amount of chlorhydrin will be hydrolyzed during the distillation which takes about four hours. The hydrochloric acid thus formed, since it forms a constant boiling mixture of maximum boiling point, would tend to remain in the flask. A study of the distribution of the ethylene chlorhydrin recovered in the 400 cc. of distillate indicates that the loss from the first source is about 3 to 4 per cent. Thus in 53 cases in which the distillate was collected in 100 cc. portions it was found that 59.5 per cent of the amount of chlorhydrin recovered was obtained with the first 100 cc. portion and 23.7, 11.4, and 5.4, respectively, in the last three 100 cc. fractions.

The total recovery of chlorhydrin was estimated by adding a known amount of chlorhydrin to cut pieces of untreated potato and distilling under the same conditions as during the regular determinations. Some distillations were also made in which an additional 300 cc. of water were used instead of the 300 grams of potato tissue. As shown in Table XI the

TABLE XI

RECOVERY OF ETHYLENE CHLORHYDRIN BY THE DISTILLATION METHOD

Amount added, cc. N/10	Per cent recovered	
	From water	With cut tubers added
54.4	92.0	85.2
54.6	86.5 91.0	88.4 87.1
54.6	91.0 90.0	84.2 81.8
Average	90.1	85.3

recovery obtained in the presence of the potato pieces averaged 85.3 per cent and with water alone 90.1 per cent. In these tests the chlorhydrin was of course added to the solution and the conditions were not the same as if the chlorhydrin were contained within the tubers. However, a study of the distribution in the four fractions of the chlorhydrin recovered in cases in which the chlorhydrin was contained within the tubers as compared with the cases in which the chlorhydrin was added directly to the mixture indicates no essential difference. The pieces of potato tuber, weighing 2 grams each, give up their chlorhydrin to the water surrounding them quite readily.

When dormant tubers are used the pH increase that may result from treatments with ethylene chlorhydrin must be taken into account. Experiments which have been conducted since the tests reported in this paper were carried out have shown the pH of the distillation mixture is a factor in the recovery of the chlorhydrin.

The ethylene chlorhydrin content of non-dormant tubers of the Green Mountain variety which had been exposed to the vapor from 5 cc. of the 40 per cent solution in a container of 17.5-liters capacity for four days is shown in Table XII. Duplicate treatments were started on each of four days. In the case of experiment 3 duplicate analyses of the same treatment were also made.

TABLE XII

ETHYLENE CHLORHYDRIN CONTENT OF TUBERS AFTER EXPOSURE TO THE VAPOR FOR FOUR DAYS (DUPLICATE TREATMENTS)

Exp. No.	Mg. per 100 g. tissue	
1	166	139
2	158	150
3	136 137	129 125
4	126	136

Determination of the chlorhydrin content of treated tubers at intervals after the end of the treatment period showed that the chlorhydrin content decreases quite rapidly with time. Although it was known that some chlorhydrin is given off by the tubers after treatment, the decrease in chlorhydrin is much greater than can be accounted for in this way. Experiments were conducted in which tubers which had taken up various amounts of chlorhydrin as a result of exposure to the vapor were stored under conditions in which the chlorhydrin given off unaltered was determined as well as the chlorhydrin remaining after various intervals. The data are shown in Table XIII. During five to eight days after the end of the treatment period the amount of chlorhydrin which could be recovered

TABLE XIII
DECOMPOSITION OF ETHYLENE CHLORHYDRIN IN POTATO TUBERS

Duration of treatment, days	Ethylene chlorhydrin, mg. per 100 g. tissue					
	Content at end of treatment	Vapor from intact tubers after treatment: Hours	Vapor from intact tubers after treatment: Mg. per 100 g.	Content after this interval	Amount lost	Amount decomposed in tubers per hour
1	201	142	28	75	126	0.68
1	178	142	15	48	130	0.81
2	299	166	24	184	115	0.55
2	179	166	15	105	74	0.35
4	272	113	13	208	64	0.45
4	158	113	9	43	115	0.93
1	82	119	8	28	54	0.39
1	41	119	4	<1	41	0.31
1	82	189	8	5	77	0.36

from the tubers decreased rapidly (column 6). The loss accounted for by the vapor given off by the tubers is shown in column 4. In the most extreme case this amounted to only 22 per cent of the decrease in the chlorhydrin content. The last column shows the decrease in chlorhydrin attributed to decomposition of the chemical in the tubers. In these tests this varied from 0.31 to 0.93 mg. of chlorhydrin per 100 g. tissue per hour.

This rather rapid decrease in the chlorhydrin content is very interesting and it is proposed to study this change thoroughly with dormant tubers. It should be possible to determine what relationship exists between the dormancy-breaking activity and the amount of chlorhydrin taken up and the quantity decomposed. An attempt will be made to determine how much chlorhydrin is necessary to insure the breaking of dormancy. Experiments can then show under what treatment conditions the absorption of these amounts is most efficiently brought about. Experiments will also be conducted to find out what relationship exists between the uptake of chlorhydrin and its decomposition and the rise in respiration which takes place soon after the exposure of dormant tubers to the vapor (14).

Determination of the chlorhydrin content of tubers after a period of treatment, especially if this is rather extended, is thus not a measure of the chlorhydrin taken up but rather an estimation of the difference between the amount taken up and the amount decomposed. A number of experiments in which tubers were treated at various temperatures from 5° to 35° for four days showed little difference between the amounts present at the end of the treatment period at the different temperatures. The probable slower rate of absorption at the lower temperatures was thus apparently balanced by a correspondingly slower rate of decomposition at these temperatures. These results are of interest in relation to the data

previously presented showing the small effect of temperature of treatment on the dormancy-breaking action. Tests in which the chlorhydrin content of tubers was determined at the end of one, two, and four-day treatments in a comparable series often showed a content at two days as great as that at four days. This result may be related to the balance between uptake and decomposition.

EFFECT OF CHEMICALS OTHER THAN ETHYLENE CHLORHYDRIN

The results indicate that probably at least 5 cc. of 40 per cent ethylene chlorhydrin per 30 l. of air space are required (at least if the air is not stirred), and attention was turned toward finding a chemical that would be effective in lower concentrations.

Since both ethylene chlorhydrin ($ClCH_2CH_2OH$) and sodium thiocyanate ($NaSCN$) are effective the compound ethylene chlorthiocyanate ($ClCH_2CH_2SCN$) was prepared from ethylene chlorobromide and potassium thiocyanate as described in Beilstein (1, p. 176). The product was purified by distillation in vacuum. Analysis for sulphur gave 26.48 per cent and the per cent S in $ClCH_2CH_2SCN$ is 26.37. This chemical was effective at about one-tenth or one-twentieth of the concentration of the 40 per cent ethylene chlorhydrin. Further tests will be made as to the range of concentration of the chemical and stages of dormancy of the tubers within which successful forcing can be obtained. Other chemicals which hastened sprouting when used at concentrations lower than that required for ethylene chlorhydrin were: epichlorhydrin $ClCH_2CH \cdot O \cdot CH_2$; mesityl oxide $(CH_3)_2C{:}CH \cdot CO \cdot CH_3$; furane $(CH)_4O$; chloracetal $CH_2ClCH(O \cdot C_2H_5)_2$; and paradichlorbenzene ($C_6H_4Cl_2$).

DISCUSSION

The results showing that even after a period of high temperature storage a treatment with chemicals is effective in hastening germination should find application in localities in which the long storage periods needed by this method can not be used. The length of the preliminary storage period can be materially shortened by the use of chemicals. Furthermore, when the high temperatures that are needed can not be obtained or can not be maintained the chemical treatments will be particularly helpful since the temperature of storage previous to the application of the chemical treatment is not a critical factor.

The cause of the unfavorable results reported by certain other investigators on the use of sodium thiocyanate was not found. Instead the experiments showed a rather wide range of concentration of chemical, and of temperature after planting within which good sprouting of dormant tubers and avoidance of injury to non-dormant tubers were obtained.

Although, as stated above, certain investigators have reported un-

favorably on the use of sodium thiocyanate, Stuart and Milstead (21) tested various varieties under different conditions and show clearly that treatment with NaSCN is effective in breaking dormancy. Simon (19) in Czechoslovakia, also, reports favorably on the use of sodium thiocyanate with dormant potato tubers.

The experiments showed that a concentration of vapors of ethylene chlorhydrin can be obtained which when maintained for seven days will break the dormancy of potato tubers and will induce the initial stages of germination to such an extent that when the tubers are cut and planted two weeks after treatment satisfactory germination both as to promptness and percentage will be obtained. The particular amounts of chemicals used in these small scale tests may not be the most satisfactory ones for treatment of larger amounts of tubers in large rooms. The completeness of evaporation of the amount of chemical which is placed within the space will depend certainly upon the temperature and probably upon whether the air is stirred and if it is stirred, upon the rate of stirring. Also the measurements show that the potato tissue takes up an appreciable amount of the chemical and consequently that the amount of potatoes in the process of treatment will be a factor in determining the most favorable amount of chemical to use. Finally, since rooms will vary in the rate of loss of vapors by leakage it seems that the most favorable amount of chemical to use in a given space in which the treatments are to be applied can be determined only by actual tests under practical conditions.

The results showing that tubers planted 3 days and those planted 14 days after treatment sprouted at nearly the same time counting from the end of the period of treatment show that tubers could be treated either in the North and shipped to the South, or they could be shipped to the South first and treated there. It is possible that further experiments will show that if the tubers are allowed a period of rest during shipment from the North to the South, the dormancy may be broken more easily at that time, and that the treatments may be applied over a wider range of temperature conditions and chemical concentration.

SUMMARY

1. Potato tubers cut into pieces ready for planting and soaked for one hour in 1.0 per cent sodium thiocyanate solution were planted in soil in flats which were placed at three different temperatures (room temperature, 29° C., and 35° C.). Favorable results were obtained at all temperatures; dormant tubers were forced into prompt germination; non-dormant tubers were not materially delayed in sprouting; and decay of seed pieces did not follow this treatment in any case. A 0.5 per cent solution of NaSCN under these conditions gave similar results except that a somewhat less favorable effect upon the germination of dormant potatoes was obtained.

A 2.0 per cent solution hastened germination but caused rotting of seed pieces in 7 out of 24 tests.

2. At intervals of 7, 14, and 28 days after harvest potato tubers were stored at 35° C. for periods of 10, 18, and 30 days at the end of which periods some lots of the stored tubers were planted without treatment, while other lots were given chemical treatments using ethylene chlorhydrin and sodium thiocyanate. Treatments with either of these chemicals hastened the germination of the tubers previously stored for the different periods at 35° C., provided that the preliminary storage period at 35° had not been long enough to cause injury to the tubers.

3. Whole tubers exposed to vapors of ethylene chlorhydrin for 7 days were stored in bags in air for 14 days following treatment. Visible sprouting occurred in about 7 to 10 days after the end of the period of treatment and well-developed sprouts were obtained with the treated tubers at the end of the period of storage. The treated tubers were cut into pieces and planted; a high percentage of sprouts appeared above ground about 20 to 30 days afterwards. These results were obtained even when there was a considerable variation in temperature during the period of treatment, during the period of storage, and after planting in the soil. This indicates that it may be possible to develop a procedure for the pre-treatment of tubers in the North shortly after harvest in late summer or early autumn for distance shipping to localities in the South in which the treated tubers are to be planted.

4. The vapor pressure curves for ethylene chlorhydrin and for its constant boiling mixture from room temperature to the boiling temperature were determined.

5. Details of a method for determining the chlorhydrin content of treated potato tubers by recovering it by distillation and subsequently forming $BaCl_2$ through reaction with $Ba(OH)_2$ are given. Recovery is about 85 per cent.

6. Potato tubers, when exposed to the vapor of ethylene chlorhydrin under conditions which have been found to break dormancy, take up relatively large amounts of the chemical. Quantities up to 300 milligrams per 100 g. of tissue have been recovered from treated non-dormant tubers.

7. The chlorhydrin content of tubers after treatment gradually becomes less. A certain amount is given off as vapor for the first few days; a larger amount is decomposed in the tissue. With non-dormant tubers the quantity lost and not accounted for by the vapor given off by the tubers was about 0.50 mg. per 100 g. of potato tubers per hour for the first week after the end of treatment.

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A STUDY OF PEACH YELLOWS AND ITS INSECT VECTOR

ALBERT HARTZELL

Peach yellows, an insect-borne disease of the peach (*Prunus persica* [L.] Stokes) and related plants, is confined to eastern temperate North America. It has been known as a distinct disease for approximately 150 years and ranks as one of the most important diseases throughout the area in which it is endemic. The present investigation reports results of experimental work[1] extending over a number of years with special reference to the relation of insects to the spread of the disease and the study of the plum and peach leafhopper, *Macropsis trimaculata* Fitch, its insect vector.

HISTORY AND DISTRIBUTION OF THE DISEASE

The peach has been grown in eastern United States since the early part of the seventeenth century (26). In 1683 William Penn mentions that the Indians grew peaches in abundance in the Delaware River Valley. So well did the peach adapt itself to its new environment that the early settlers believed it to be indigenous. For more than one hundred years, if we may rely on statements in the literature of that period, the peach flourished. There are records of peach trees living from 40 to 70 years. Toward the latter half of the eighteenth century a decline in the longevity of the peach was observed. This was due partly to the ravages of the peach borer which first became troublesome about that time, but was no doubt due also to the spread of a new highly infectious disease called yellows.

This disease is believed to have become established in the lower Delaware Valley in the latter half of the eighteenth century. The first authentic record (24, p. 15–24) was that of Judge Richard Peters, who reported the disease as appearing on his farm in the vicinity of Philadelphia about 1791. He is credited with giving the name "yellows" to the disease. In 1796 a prize offered by the American Philosophical Society on the cause of the degeneration of the peach was divided between two contestants both associating the trouble with insects (26). The first unmistakable description of yellows was published by William Prince (25, p. 14–15) in 1828. He described for the first time the premature ripening of the fruit.

The disease seems to have terminated a profitable peach industry in southeastern Pennsylvania, New Jersey, and Delaware. It was reported (26) from New York during the first decade of the nineteenth century, from Connecticut in 1814, from Massachusetts in 1854, from Michigan in

[1] The writer is indebted to W. A. McCubbin and T. L. Guyton for the privilege of the facilities of the Bureau of Plant Industry of the Pennsylvania Department of Agriculture during the course of this investigation, and to Albert Miller of Cornell University for assistance in some of the experimental work.

1866, and from Ontario, Canada in 1878. The distribution of the disease has not markedly changed during the last fifty years. It is known to occur in Ohio, Indiana, Kentucky, and was reported from Illinois (33) in 1927. From Virginia its range extends southward along the Appalachian Mountains with occasional outbreaks in Tennessee and the Carolinas (2). Sporadic outbreaks have occurred in Iowa, Missouri, Arkansas, northern Texas, and Nevada, but the disease has never become established west of the Mississippi River (2, 27, 30), nor have there been authentic reports from the extensive peach growing districts of Georgia and California.

The distribution of peach yellows is, therefore, not coincident with the peach growing regions of the United States but is confined to the eastern temperate North America. So far as it is known the disease has not become established in any other part of the world.

The losses caused by peach yellows during epidemic years have at times brought the peach industry to a low ebb. Smith (26) reports as high as 50 per cent of the trees infected in individual orchards in a single year. The greatest incidence that the writer has ever observed in one year was 33 per cent in a small orchard near a tract of woodland at Pricetown, Pennsylvania.

Great outbreaks of yellows (26) occurred in 1791, 1806–1807, 1817–1821, 1845–1858, 1874–1878, 1886–1888, and 1920. These periods were followed by years of comparative immunity.

SUSCEPTS

Yellows is limited to the peach, nectarine (*Prunus persica* [L.] Stokes var. *nucipersica* Schneid.), almond (*Prunus communis* Fritsch), apricot (*Prunus armeniaca* L.), and Japanese plum (*Prunus salicina* Lindl.) (26, 30). Manns[2] (20) believes that the cultivated plum (*Prunus domestica* L.) is not immune but the symptoms are masked.

There is no evidence that one variety of peach is more susceptible to yellows than another variety. The disease occurs in seedlings. Seedlings grown from Asiatic seed are not immune to yellows (1). Under orchard conditions trees under five years rarely become diseased. McCubbin (16) found from a study of inspection records in Pennsylvania that the incidence of the disease increased from 0.002 per cent for trees two years old to 0.939 per cent for 10-year-old trees.

SYMPTOMATOLOGY

The presence of witches' broom and the premature ripening of the fruit are considered to be definite symptoms of peach yellows by all investi-

[2] Since the above was written, the following has been published by T. F. MANNS and M. M. MANNS, "Plums as factors in the dissemination of yellows and little peach" in Trans. Peninsula Hort. Soc. **48**: 72–76. 1934 (1935).

gators who have made a careful study of the disease. Premature ripening of the fruit from a few days to three weeks is recognized as the most definite symptom. The skin of the peach is highly colored, spotted with red and purple, and the flesh marbled with crimson with pronounced coloring around the pit. The fruit is apt to be large but is of an inferior quality and the taste bitter. Such premature fruit may be borne on a single branch or the situation may be reversed, with the fruit all premature with the exception of a single branch which may bear normal-appearing fruit. The second year the fruit is apt to be smaller than normal.

Premature unfolding of leaf buds is another characteristic of the disease. These develop into willowed shoots in which the terminal buds are not dormant, resulting in a wiry broom-like growth bearing vary narrow yellowish leaves which are often spotted with red and continue to grow after normal leaf fall. Twigs arising from the lower side of a branch have a tendency to grow vertically (Fig. 1 A) in place of forming a wide angle as is the case with normal branches. The same tendency to grow vertically is seen in shoots on the terminals of the branches. These appear on the larger limbs and may or may not be associated with premature fruit, and are recognized as a definite symptom of yellows. When diseased trees were grown in an inverted position the willowed shoots showed the same tendency to be negatively geotropic (Fig. 1 B) as twigs of normal trees and retained the same relative growth angle. There is a tendency for the diseased leaves to roll and to droop toward the branches and trunk.

It has been observed that affected trees set their buds earlier in the fall and that these are well developed when winter comes. In the spring both the flower buds and leaf buds develop earlier than in healthy trees. This is especially noticeable when a single branch of a tree is diseased. The affected branch will be in full bloom while the fruit buds in the remainder of the tree are undeveloped. Occasionally diseased trees bloom in the fall.

In young trees one of the first symptoms to be noticed is the failure of the latent buds to remain dormant. These unfold into yellowish leaves scarcely more than an inch in length giving the tree a bushy appearance. The larger leaves may be mottled with areas of dark and light green (Fig. 1 C and D). The terminal twig also has a tendency to grow vertically. Young diseased trees grow upright in contrast with the spreading habit of normal trees. In older trees the leaves have a tendency to curl slightly and droop and are of a yellowish-bronze color in contrast with the normal green foliage of healthy leaves, which has doubtless suggested the name of yellows. The symptom becomes more pronounced as the disease progresses and the leaves show a claw-like curling. This is followed by the death of the ends of the large limbs with clusters of short twigs bearing sparse yellowish foliage (Fig. 2 A).

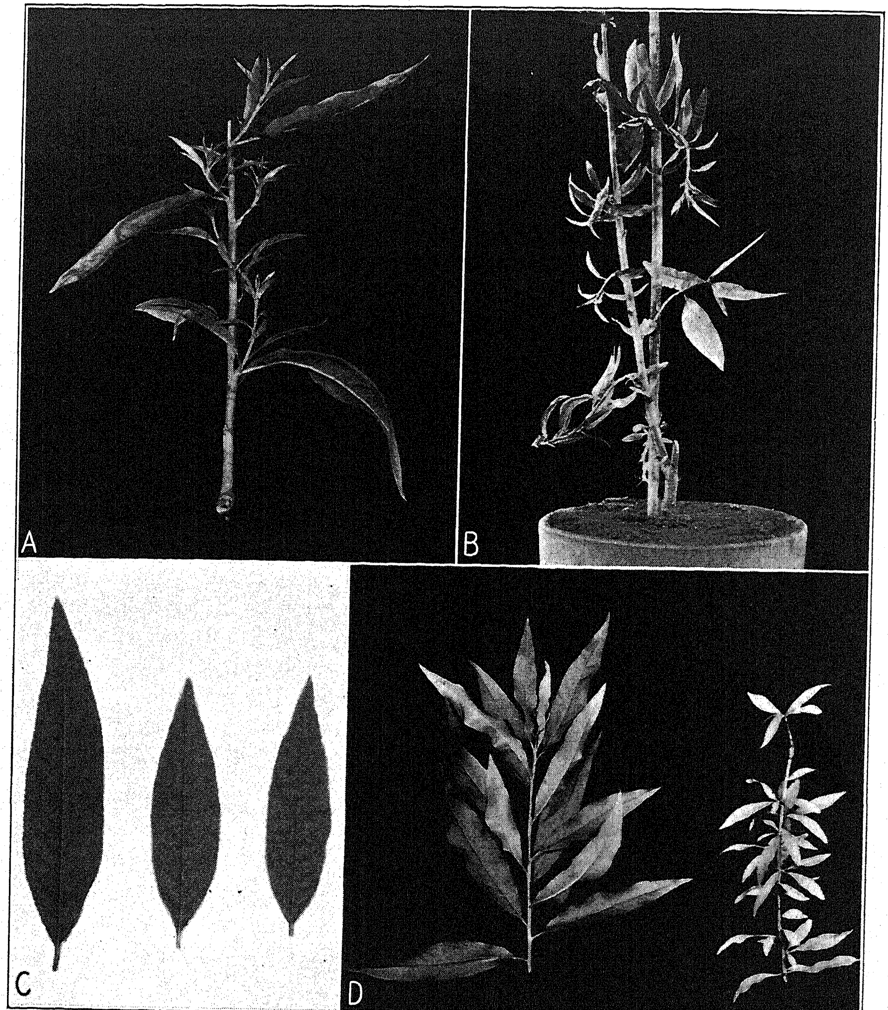


FIGURE 1. Symptoms of peach yellows. A. Branch showing typical diseased shoots. Note that the shoots fail to form a normal growth angle with the branch. B. Diseased seedling that was grown in an inverted position. Note that the shoots retain the same relative growth angle with the branch as in (A) but that they extend in the opposite direction. C. Leaf from healthy tree at left. Two leaves from diseased tree showing mottling at right. Note difference in size. D. Healthy twig at left showing normal leaf development. At right, twig from diseased tree to which peach yellows was transmitted by *Macropsis trimaculata*. Note difference in size of leaves and the large number of leaves that developed from an equal length of stem with consequent shortening of internodes.

FIGURE 2. Transmission of the disease. A. Seven-year-old peach tree in last stage of the disease from natural spread. Note dead branches at top. B. Yellows developing on seedling that was budded with diseased bud two years previously. C. A row of peach trees that was budded with scions from diseased trees, as it appeared two years after budding. The trees in the left half of the row were budded with normal-appearing buds from diseased trees. The trees in the right half of the row were budded with scions from twigs showing positive symptoms of the disease. Note that these trees are stunted.

The fact that the symptoms progress from part to part until the whole tree is involved lent favor to the idea that the disease is localized and not systemic. That this is not the case is proved by the failure of excision of diseased branches to arrest the spread of the disease throughout the tree (27).

OTHER INJURIES MISTAKEN FOR YELLOWS

Unfortunately a number of troubles produce symptoms similar to yellows such as winter injury, mechanical injuries of various kinds, starvation, borer injuries, etc. Trees affected with yellows can be readily distinguished from borer infestation injuries by the tenacity with which the leaves adhere to the twigs. In the case of a tree infested with borers the leaves are easily dislodged while the leaves of trees affected with the yellows disease adhere more firmly to the twigs.

Little peach is considered to be a distinct disease from yellows. The most trustworthy symptom of the former is the delayed ripening of the fruit in contrast with the premature ripening of fruit on trees affected with yellows.

ETIOLOGY

In the past yellows has been attributed to many different causes (29). Winter injury, the direct effect of various species of insects and mites, and physiological disturbances brought about by deficiency in nutrient supply, such as potassium and chlorine, have all been suspected as the cause of the disease. When the germ theory of disease was generally accepted attempts were made to connect yellows with a specific bacterium or fungus (4, 6). At present yellows is usually classified as a virus disease because of certain similarities between it and the mosaic disease, notably that it is transmissible by grafting, budding, and tissue implanting, and that no organism has been found associated with it. Some investigators regard its cause as a non-living chemical entity possibly enzymatic in nature (7). That yellows is a filterable virus lacks positive proof in that no one has successfully transferred the virus from a diseased to a healthy plant by artificial inoculation. The virus appears to be transmitted only when the tissue of a diseased plant is caused to unite with the healthy plant (26); mere contact does not suffice to transmit the disease. Aside from insect transmission this is the only method known.

UNSUCCESSFUL MEANS OF TRANSMISSION OF THE DISEASE

Pollen. The observation that the disease first appears on the fruit suggests transmission by pollen. Prince (25) believed that the pollen from diseased trees was capable of transmitting the disease when blown by the wind to the flowers of healthy trees. This view is partly negated by the

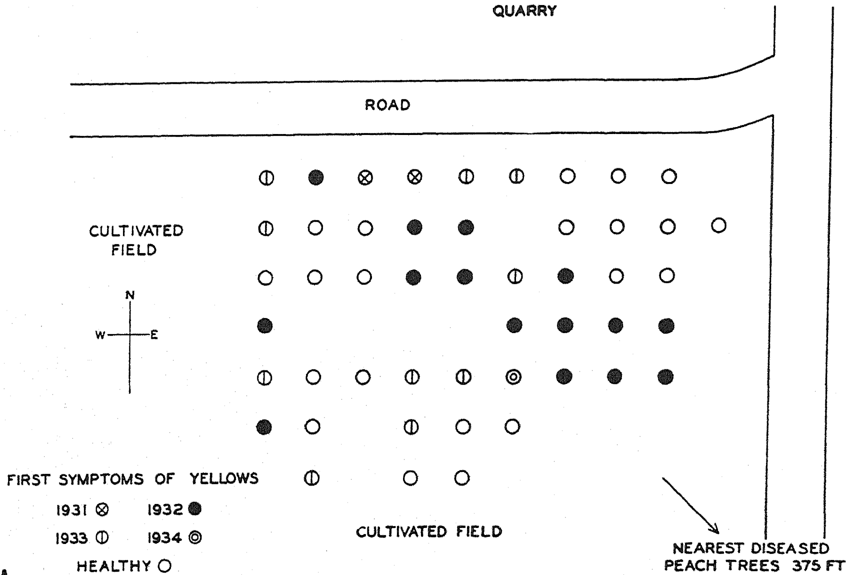
fact that a number of varieties of peach have been proved to be capable of self-pollination (1, p. 150–153). Blake, Cook, and Connors (2) pollinated a number of trees with pollen from diseased trees. They state that the pollen was viable, did not transmit the disease to the healthy trees, and that the seed resulting from these crosses produced healthy trees.

In the present investigation approximately half of the trees in a small experimental peach orchard were set aside for pollination tests. The remainder of the trees were held as checks. The trees were five years old and appeared to be healthy. The varieties represented were Elberta, Carman, Stevens, Rochester, and Early Crawford. The previous season two diseased trees had been removed from this orchard. In order to prevent pollination by insects five trees were caged with unbleached cheesecloth before the flower buds opened and the cages were not removed until the fruit had set.

Approximately 100 blossoms on one limb of each of 12 trees, including 4 caged trees, were brush-pollinated with pollen collected from diseased trees. All flowers that were not open were removed from the limb, which was labeled. The pollen had been collected 24 hours previously and allowed to dry at room temperature.

In order the prevent self-pollination a series of flower buds were emasculated, selecting trees on which there were no open flowers, and all buds that were not emasculated were removed from the branch. In this series were eight trees including two caged trees. As soon as the flowers of a branch had been emasculated they were sacked and labeled. When the bees were active in the orchard and the stigmas of the emasculated flowers showed secretion, the flowers were brush-pollinated with pollen collected from diseased trees as described above. The branches were resacked until the fruit set, when all the sacks were removed. The checks consisted of 22 trees scattered in checker-board fashion throughout the orchard, which were not pollinated artificially. The incidence of yellows was high in the orchard during 1932 and 1933 while this experiment was in progress (Fig. 3).

It will be noted from Table I that there was no positive correlation between any series of the pollinated and checks. At the end of the first growing season (1932) a total of four trees in the not-emasculated series showed symptoms of yellows while eight were healthy. In the emasculated series three trees showed symptoms of yellows while five remained healthy. In the checks six trees became diseased while 16 remained healthy. There was no positive correlation between limbs pollinated with diseased pollen and the incidence of the disease. If we include the second year's (1933) results we have almost a perfect case of independence between the incidence of yellows and pollination with diseased pollen. Considering the



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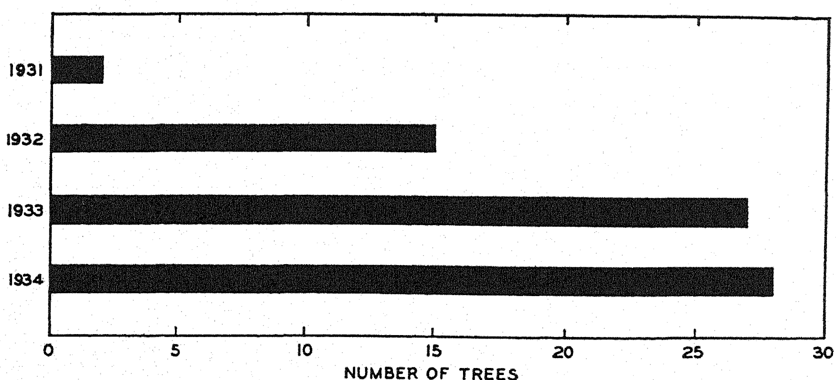


B

FIGURE 3. A. Natural spread of yellows in experimental peach orchard. B. Incidence of peach yellows in experimental peach orchard showing number of diseased trees by years.

TABLE I
POLLINATION TESTS IN EXPERIMENTAL PEACH ORCHARD

	Brush-pollinated with pollen from diseased trees												Checks			
	Flowers not emasculated						Flowers emasculated									
	Trees that became diseased				Healthy		Trees that became diseased				Healthy		Trees that became diseased		Healthy	
	Caged		Not caged				Caged		Not caged							
	Limb pol.	Limb not pol.	Limb pol.	Limb not pol.	Caged	Not caged	Limb pol.	Limb not pol.	Limb pol.	Limb not pol.	Caged	Not caged	Caged	Not caged	Caged	Not caged
	1	1	2	—	0	8	—	1	1	1	0	5	0	6	1	15
	2		2		0	8	1		2		0	5	0	6	1	15
1932	4				8		3				5		6		16	
1933	5				7		4				4		11		11	

number of checks that became diseased, the results in order to be significant required that 12 trees become disease in the treated the first year, whereas only 7 trees became diseased. With a total of 11 check trees diseased for the second year (1933), it would require 16 of the treated trees to become diseased in order to be significant, whereas only 9 trees had become diseased. It is, therefore, concluded that pollination of healthy trees with diseased pollen does not increase the incidence of peach yellows.

Seed. Opinions differ as to whether yellows is transmitted through the seed. According to Smith (26), La Fleur, a Michigan grower, claims to have obtained diseased seedlings from pits taken from diseased trees. Negative results have been reported by Smith (26), Caesar (5), Blake, Cook, and Connors (2), and McCubbin (15). In several instances more than a thousand seeds were used. The germination ranged from 0.5 to 8.0 per cent and all the pits germinated by these investigators produced healthy seedlings. In the present investigation several attempts were made to germinate small lots of seed from diseased peach trees without success.

Mechanical inoculation. That yellows is not spread by mechanical injuries is substantiated by the fact that injuries incident to pruning and cultivation of the peach do not infect the trees (26). Many attempts have been made to transmit the disease by mechanical inoculation with sap and the juice obtained from pressed bark, twigs, flowers, leaves, and fruit (2, 5, 18, 22). All these attempts have resulted negatively.

The results of field experiments on artificial inoculation appear in Table II. Inoculations were made with juice extracted from diseased leaves, stems, roots, and flowers. These included transfers of the inoculum from leaf to leaf, leaf and stem to leaf, root to root, root to leaf, flower to flower, flower to twig, and flower to leaf.

The inoculum was prepared by macerating the diseased parts in distilled water or saliva. Other extracts were made by subjecting the diseased tissue to a hydraulic press and drawing off the juice into a small amount of distilled water. Inoculations were made with insect pins (No. 0) or by scratching with a dissecting needle. More than 100 trees ranging in age from 1 to 7 years were inoculated, but all remained healthy for the periods, ranging from 1 to 4 years, that the trees were kept under observation after inoculation.

Non-vector insects. Kunkel (13) lists 14 species of insects that failed to transmit peach yellows. In addition Manns (19, 20, 21) reports several species that gave negative results.

In this investigation the writer has tested 47 species of insects and mites as possible vectors of peach yellows that failed to transmit the disease of which 20 species were present in sufficient number to be care-

TABLE II
FAILURE TO TRANSMIT PEACH YELLOWS BY MEANS OF MECHANICAL INOCULATION

Parts of diseased peach tree from which noculum was obtained	Manner of extraction of inoculum	Medium	Method of inoculation	Number inoculated									
				Flowers	Leaves		Twigs	Roots	Branches	No. trees	Tree age, yrs.	Date of inoculation
					No.	No. punctures per leaf						
Leaves and stems	Macerated	Dis. water	Needle	—	9	100	—	—	1	1	1	July 18
	Hydraulic press	Dis. water	Needle	—	100	100	—	—	4	1	4	Sept. 25
Leaves	Hydraulic press	Dis. water	Scratching	—	3	100	—	—	—	100	2	Aug. 17
Roots	Hydraulic press	Dis. water	Needle	—	—	—	—	*	—	1	4	Sept. 28
	Hydraulic press	Dis. water	Needle	—	100	100	—	—	4	1	4	Sept. 28
Flowers	Macerated	Saliva	Needle	100	—	—	—	—	1	1	7	May 10
	Macerated	Saliva	Needle	—	—	—	6	—	1	1	7	May 10
	Macerated	Saliva	Needle	—	50	—	—	—	1	1	7	May 10

* 1000 punctures on many rootlets.

fully tested. In all 925 trees ranging in age from 1 to 4 years were exposed.

Two hundred one-year-old trees were exposed to the green peach aphid (*Myzus persicae* Sulzer) that had been reared on diseased trees. The healthy seedlings were infested with 10 to 50 insects each for periods of 10 to 60 days. These tests were made over a period of three years. This species is found throughout the peach growing region of United States and Canada. It is our most common peach aphid.

The black peach aphid (*Anuraphis persicae-niger* Smith) was selected for study as a possible carrier of peach yellows because its geographical range is practically coextensive with that of the disease. Its tendency to occur in definite localities that are widely separated is similar to the occurrence of yellows. Normally it feeds on the roots but it occasionally feeds above the ground. It is interesting to note that the botanist, Erwin F. Smith (26), and the entomologist, John B. Smith (31), were led to think for a time that this species was the direct cause of yellows. During a period of two years 260 healthy peach trees ranging in age from 1 to 4 years were exposed to agamic forms of this species that had fed from 2 to 10 days on diseased trees. Repeated infestations with 5 to 10 insects per tree were made at weekly intervals throughout the growing season. A limited number of tests were made with six unidentified species of aphids that fed on peach.

Transmission tests involving 10 to 100 individual insects were made with the following species of leafhoppers both as nymphs and adults: *Graphocephala coccinae* Forst., *Jassus olitorius* Say, *Erythroneura obliqua* Say, *Erythroneura comes* Say, *Ormenis pruinosa* Say, *Acanalonia bivittata* Say, *Empoa rosae* L., *Eutettix strobi* Fitch, *Empoasca fabae* Harris, *Empoasca* sp., *Cicadula sexnotata* Fall., and *Agallia sanguinolenta* Prov. Tests involving fewer individuals were made with eight other species of leafhoppers.

Adults of the following additional sucking insects were tested as possible vectors of the disease: *Ceresa bubalus* Buckt., *Lygus pratensis* L., *Poecilocapsus lineatus* Fabr., and *Tibicina septendecim* L. Among this group were several unidentified Mirids and Psyllids.

Thrips tabaci Lind. and *Heliothrips femoralis* Reuter were tested both as larvae and adults.

The mites tested included *Tetranychus telarius* L., *Tarsonemus pallidus* Bks., and an unidentified species of *Eryophyes*.

In addition the plum curculio (*Conotrachelus nenuphar* Herbst.) and the peach borer (*Aegeria exitiosa* Say) were tested as vectors. A number of unidentified species of Lepidoptera and Coleoptera found feeding on peach were included in the transmission tests.

The writer is indebted to Dr. Edith M. Patch for the identification of aphids. Leafhopper specimens were sent to Professor Herbert Osborn

who kindly identified a number of species listed above or furnished identified material with which the specimens were compared.

TRANSMISSION OF THE DISEASE

Transmission by Budding

According to Smith (26), Robert Sinclair was the first to present experimental evidence that peach yellows is transmissible to peach by means of diseased buds. The disease is said also to be transmissible by grafting and tissue implanting (26). Smith (27, 28) reports a series of experiments in which nearly a thousand peach trees were budded with diseased buds. In one lot of 202 trees budded in the fall he obtained transmission of the disease from the bud to the stock in 40 per cent of the cases, the symptoms appearing in 14 months. In another lot of 200 trees only 39 were healthy the following November. Similar results have been reported by other investigators (2). There appears to be a marked difference in the virility of buds to transmit the disease. In one instance Smith (27, 28) budded a series of trees with apparently healthy buds taken from diseased trees. The incubation period was greatly prolonged. After two years only 3 trees were healthy, 16 were doubtful, 103 were definitely diseased, 88 were dead, nearly all from yellows. Of the checks 117 were healthy, 1 was doubtful, and 8 were dead. Three years after the experiment was begun 15 of the budded trees were still living and all had yellows, while only 1 case of yellows developed in the checks. Blake, Cook, and Connors (2) have shown that there is a marked difference in virility with the buds from the same tree to transmit the disease and that the symptoms may be delayed for as long a period as three years.

The writer performed a similar experiment. A row of four-month-old trees were budded at the ground level with diseased buds in August 1929. At one end of the row 28 trees were budded with normal-looking buds from diseased trees, while at the same time 20 trees at the other end of the row were budded with scions taken from willowed shoots of diseased trees (Fig. 2 B and C). Three years later 21 trees of the first mentioned 28 had become diseased, while all the trees at the other end of the row had shown symptoms of the disease from one year to two years in advance of the other trees, only a single living tree having survived. Check rows of trees on either side remained healthy. As this experiment was concluded prior to any natural spread of yellows in our experimental plots, the results are believed to be significant.

In the course of this investigation the writer has transmitted yellows by budding to approximately 300 trees varying in age from less than one year to three years. The minimum length of the incubation period under field conditions ranged from 12 months to 3 years. In every case where

the diseased scion had united with the healthy stock and the tree survived it became diseased.

Diseased buds are capable of transmitting the virus to healthy trees even though the buds fail to develop (2). Kunkel (12) was able to reduce the incubation period in the greenhouse to a minimum of six weeks with vigorous young peach seedlings which were budded some distance above the ground level. He demonstrated the virus moves more quickly downward than upward and estimated that the downward movement is ten times as fast as the upward.

Transmission by Macropsis trimaculata

Kunkel (13) exposed 74 healthy peach seedlings to nymphs and adults of *Macropsis trimaculata* Fitch that fed on diseased peach trees. Of this number 7 developed the disease in periods ranging from 78 to 186 days after exposure.

Plan of transmission experiments. Nymphs and adults were collected from wild plum trees (*Prunus americana* Marsh.) growing locally and from peach trees in the experimental orchard and transferred to insect-proof cages containing diseased peach twigs and foliage. In some cases the insects were caged on the branches of a seven-year-old diseased peach tree, in other cases the diseased twigs were removed and placed in flasks containing water and these placed in cages containing the insect in a field insectary. The insects were allowed to feed for periods of from 6 to 17 days on diseased twigs and leaves when they were transferred to healthy seedlings in insect-proof cages where they fed for periods ranging from 14 to 46 days. A few nymphs collected from diseased trees were used immediately in the infection experiments. In another series potted seedlings were placed around a caged seven-year-old diseased peach tree known to be infested with *Macropsis trimaculata* for a period of two months to permit the insects to feed on them at will. The exposed trees in each series were kept separate. As the insect appears to be deleterious to young peach trees when exposed in large numbers (13) an average of approximately 10 individual insects per tree was used in these infection cages.

The cages in which the transmission tests were made were sufficiently large to permit four peach seedlings to be exposed at one time. The cages were tightly constructed with wooden frames and floors. The ends and top were covered with wire screen (50 meshes per inch) while the sides were made of panes of glass.

The peach seedlings used in the transmission tests had been grown in the greenhouse, except during the winter months when they were placed in cold frames. The trees ranged in age from 1 to 5 years. In the spring they were removed from the cold frames before the blossoms or leaves appeared and were placed in insect-proof cages covered with cheesecloth.

From time to time during the growing season trees were removed from these supply cages to an adjacent greenhouse where they were immediately placed in cages for transmission tests. The trees that remained in the supply cages were retained as checks. Frequent inspections were made to see that the cages remained insect-proof.

Upon completion of the transmission tests the trees that had been

TABLE III

PEACH YELLOWS TRANSMISSION EXPERIMENTS WITH MACROPSIS TRIMACULATA

Tree No.	Tree age, yrs.	Days insects exposed	Date tree exposed	No. insects		Days tree exposed	Date disease recorded	Approximate incubation period in tree, days
				Nymphs	Adults			
1, 2	3	7	June 9–12	22	0	21–30	—	—
3, 4	3	7–8	June 14	32	2	22	—	—
5, 6	3–4	7	June 18	19	5	18	No. 5, Mar. 13	268
7, 8	3	*	June 18	15	6	18	No. 7, July 30	42
9, 10	3–4	8	June 20	12	12	16	No. 9, Aug. 1 No. 10, Dec. 20	42 173
11, 12	3	7	June 20	14	10	16	—	—
13, 14, 15	3–5	*	June 20	24	6	16	No. 13, Aug. 1 No. 15, Aug. 1	42 42
16, 17, 18	3–4	7	June 21	33	0	15	No. 16, Mar. 13	265
19, 20	3–4	7	June 21	0	18	15	No. 20, Mar. 13	265
21, 22	3–4	*	June 21	4	10	15	—	—
23, 24	3–4	7	June 22	0	20	14	—	—
25, 26	3–4	7	June 22	18	0	14	—	—
27, 28, 29	3–5	*	June 25	0	30	15	No. 27, Dec. 20	180
30–33	1	*	June 29 July 2, 3	0	19	**	No. 30, Aug. 24	56
34–48	1	†	July 3	‡	‡	‡	—	—
49	1	11	July 3	0	7	16	—	—
50	1	11	July 3	0	8	16	Mar. 13	253
51	1	8	July 3	0	13	16	—	—
52	1	8	July 3	0	10	16	—	—
53	1	8	July 3	0	10	16	—	—
54, 55	1	7	July 6	0	10	25	—	—
56–59	1	10	July 9	0	27	22	—	—
60–63	1	10	July 9	0	28	22	No. 62, Aug. 24	46
64–67	1	*	July 10, 11	0	27	21	—	—
68–71	1	11	July 11	0	25	20	—	—
72, 73	3–4	†	July 11	‡	‡	‡	No. 73, Dec. 20	162
74, 75	1	6–7	July 12	0	7	19	—	—
76, 77	1	7–11	July 19	0	20	46	—	—
78–81	1	8–15	July 31	0	41	35	No. 81, Mar. 13	226
82–85	1	7–8	Aug. 10	0	5	24	—	—
86	1	17	Aug. 31	0	1	11	—	—
Av. for diseased	2.8	9	—	9.4		18.6	—	147
Av. for healthy	1.9	8.8	—	8.3		19.9	—	—

* Collected from yellowed trees.
** Until insects died.
† Insects allowed to feed at will on seedlings placed around caged yellowed trees.
‡ Unknown.

exposed to infected *Macropsis trimaculata* were transferred to separate insect-proof field cages until heavy frosts assured that there was little or no possibility of chance infection by insects. Both checks and treated trees were then removed to a cold greenhouse, with the exception of nine exposed trees and an equal number of checks which were placed for a period of 110 days at a temperature of 10° C. Exposed trees were removed to a warm greenhouse, while the check trees of this series were treated with ethylene chlorhydrin at the rate of 1/20 cc. per l. of air space for 20 hours to break the dormancy prior to being removed to a warm greenhouse. On December 27 the remaining trees were transferred from the cold greenhouse to a warm greenhouse to await development of the symptoms of the disease.

Results of transmission tests. The results of transmission tests with *Macropsis trimaculata* during the season of 1934 appear in Tables III and IV and in Figure 4. It will be noted that of 86 trees exposed 14 became diseased or an average of 16.3 per cent (Table IV). The time that it required the trees to produce the first symptoms of the disease was relatively

TABLE IV

SUMMARY TABLE. TRANSMISSION EXPERIMENTS WITH MACROPSIS TRIMACULATA

Classification		No. trees		Per cent trees diseased
		Exposed	Diseased	
Insect stage	Nymphs	7	1	14.2
	Adults	35	6	17.1
	Both	15	6	40.0
Av. No. insects per tree	0–4	7	0	0
	5–10	46	8	17.4
	11–21	15	3	20.0
Trees exposed	Before June 22	22	8	36.4
	June 23 to July 15	53	5	9.4
	July 16 to Sept. 1	11	1	9.1
All trees		86	14	16.3
Tree age, years	1	55	4	3.6
	3–5	31	10	32.2

long, ranging from 42 to 268 days, with an average of 147 days (Table III). The exact time that the first symptoms appear is ill-defined due to the difficulty of detecting the early symptoms of the disease so that the periods stated can be considered only as approximations. All of the 75 trees held as checks remained healthy.

The age of the tree seems to be an important factor as only 3.6 per cent of the one-year-old trees became diseased as compared with 32.2 per cent for the trees three years or older (Table IV).

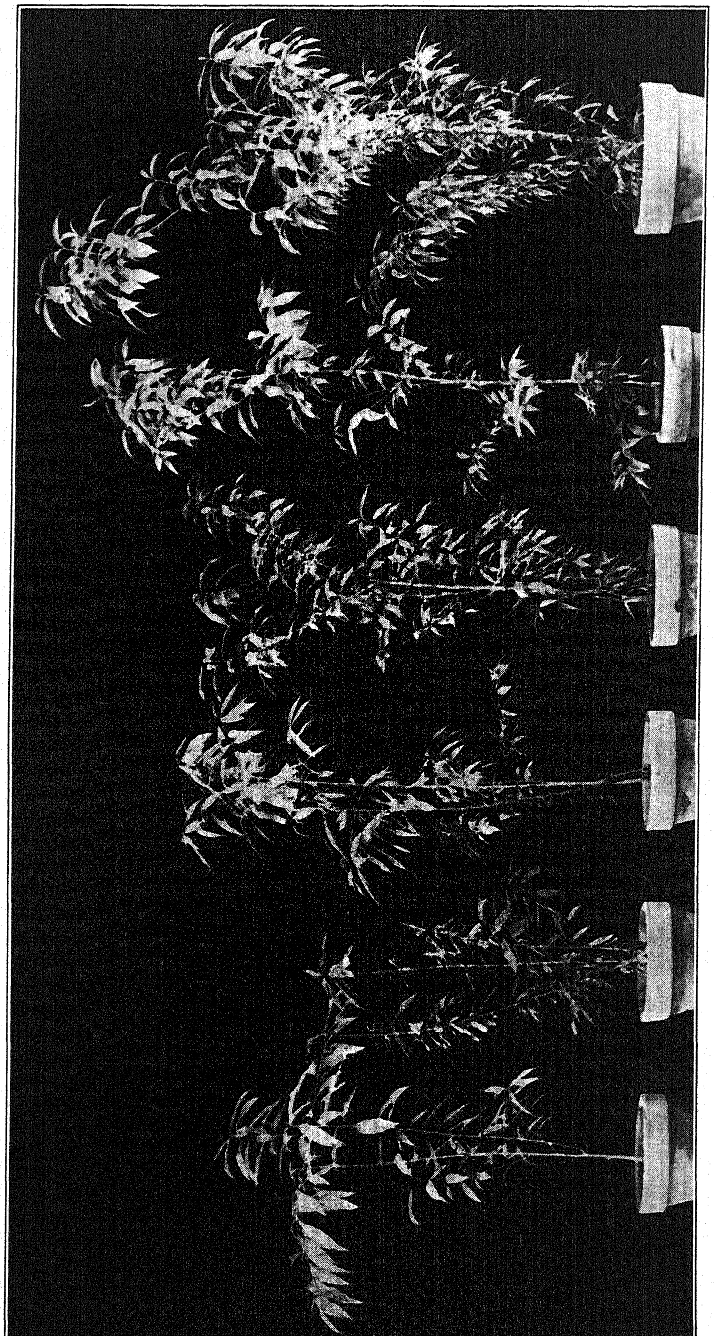


FIGURE 4. Series of five peach seedlings to which peach yellows was transmitted experimentally by *Macropsis trimaculata*. Healthy tree on the extreme left.

It will be noted that both nymphs and adults are capable of transmitting the disease. The time of the year appears also to be important as 36.4 per cent of the trees became diseased that were exposed to infected insects before June 23 while less than 10 per cent of the trees became diseased that were exposed after that date (Table IV).

While the incubation period in the insect was not precisely determined, it appears not to have exceeded 22 days for the nymphs and 32 days for the adults. When both nymphs and adults were used the average was 25 days. As a precise determination of the incubation period in the insect would have seriously interfered with the transmission experiments owing to the limited number of insects and trees available, it was decided to leave this for future investigation.

THE INSECT VECTOR

DESCRIPTION

The adults of *Macropsis trimaculata* are of a dull reddish-brown color (Fig. 5 A and B). The males are usually considerably darker and somewhat smaller than the females. The markings are rather indistinct but well marked specimens have three transparent white spots in a row on each elytron. The average length of the females is 5 mm. compared with 4.5 mm. for the males.

The nymphs are robust and are reddish-brown, with short transverse heads and broad abdomens which are lifted in a sharp crest. Each segment ends in a well defined tooth which projects backward.

For a technical description of this species see Breakey's (3) review of *Macropsis*.

LIFE HISTORY

From daily records of collections it appears that the nymphal period extends from the latter part of May to the end of the third week in June at Yonkers, New York. The first nymphs of the season were collected May 28, 1934. Nymphs collected the first week of June became adults by June 18. This would indicate a nymphal period of approximately three weeks. Adults were found in the field from the above date until August 14. In 1933 the last adults were captured August 3. Individual adults lived in cages for at least 27 days. The insect hibernates in the egg stage in the bark of wild plum. In cages egg scars were noted on peach twigs (Fig. 5 C). There appears to be a single generation a year.

Studies with individual insects were not undertaken because of the paucity of the population. Emphasis in this investigation was placed on the transmisssion experiments, which necessarily interfered with obtaining detailed life histories of the individual insects involved.

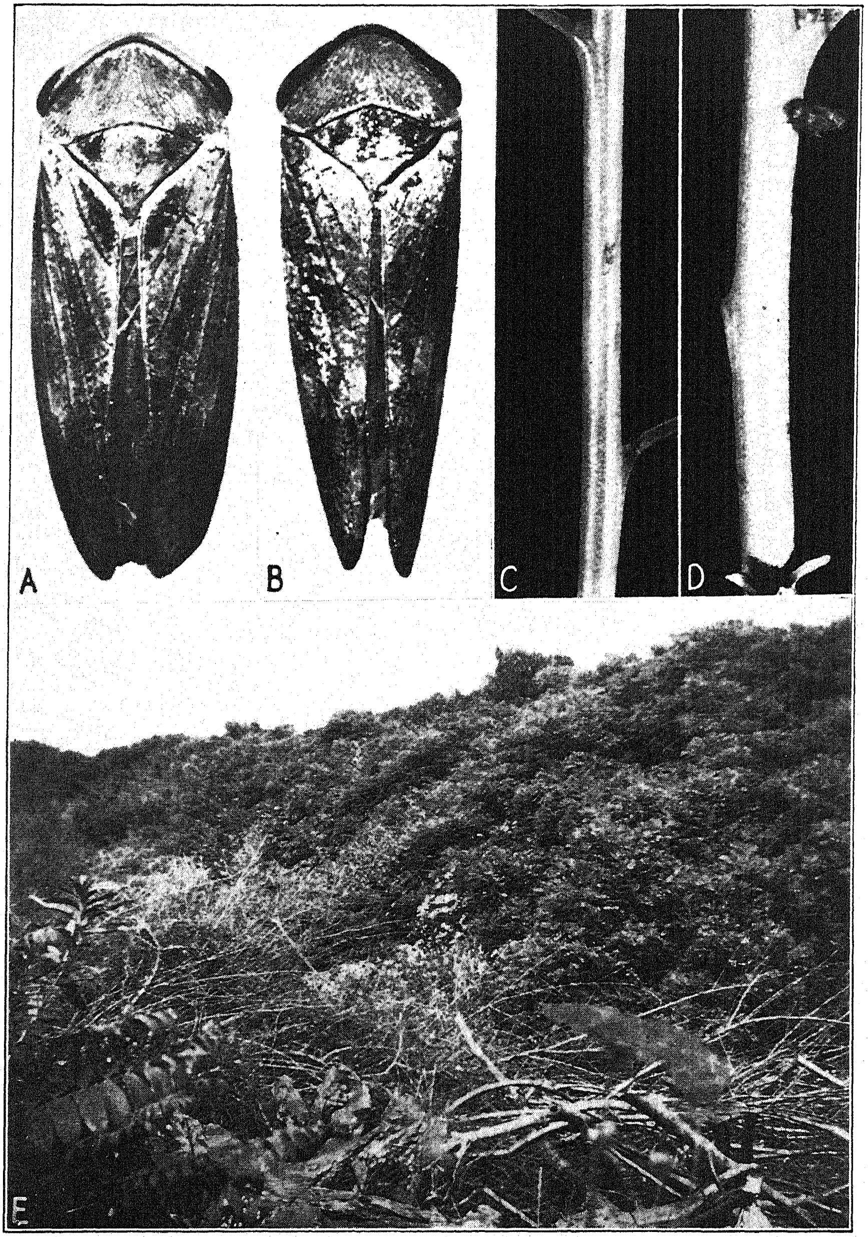


FIGURE 5. The plum and peach leafhopper, *Macropsis trimaculata*. A. Adult female. ×15. B. Adult male. ×15. C. Egg punctures. D. Feeding punctures. Note adult resting at top of twig. E. Sink hole in center of peach orchard in which all trees were healthy except for several diseased ones on the periphery of this sink hole.

HABITS

In place of feeding on foliage as is the usual habit of leafhoppers, this species seeks the twigs and smaller branches and is rarely observed on foliage (Fig. 5 D). It was observed feeding for the most part on young twigs and only occasionally on the petioles and mid-rib of the leaves. The nymphs and adults run rapidly and seek the opposite side of the limb from the observer. This combined with their color which resembles the bark of

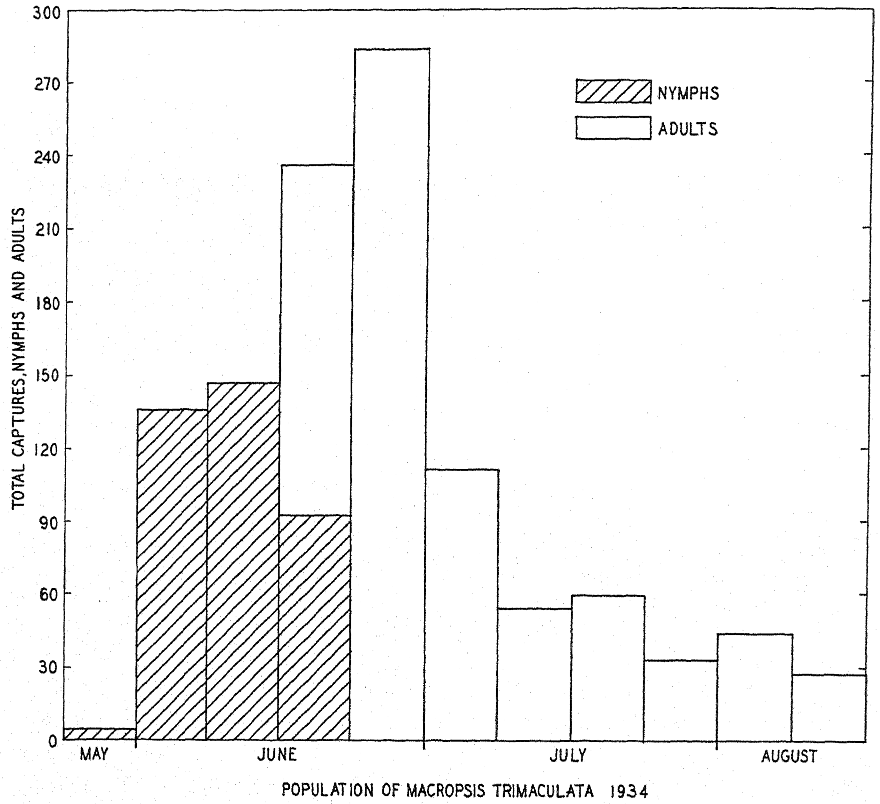


FIGURE 6. Population of *Macropsis trimaculata*, Yonkers, New York, 1934.

the tree makes them difficult to collect. They very seldom take to flight and rarely hop although they are physically able to do so when probed.

The shyness of this species, the paucity in number, combined with the fact that it is normally found on plum rather than the peach, has no doubt delayed its detection as the vector of peach yellows.

POPULATION STUDIES

Collections in the field were made daily, weather permitting, over a three-hour period throughout the growing season. The insects were col-

lected alive by means of a pneumatic insect catcher. Most of the captures were taken from wild plum but collections were made also from peach trees. The number of nymphs collected ranged from 4 during the last week of May to 147 during the second week of June. The adults reached their maximum during the last week of June when 284 were taken. They then declined very rapidly as indicated in Figure 6, but could be found sparingly until the middle of August.

As compared with other leafhoppers of economic importance *Macropsis trimaculata* is a rare species. The writer has collected a greater number of adults of *Empoasca fabae* in the potato fields of Iowa in a half hour than *Macropsis trimaculata* that could be collected during a whole season.

RELATIONSHIP OF INSECT VECTOR, WILD PLUM, AND INCIDENCE OF PEACH YELLOWS

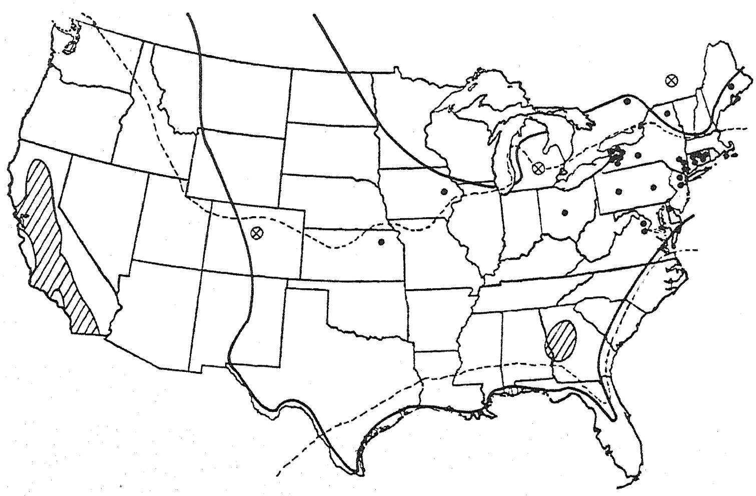
Smith (29) noted that there was a higher incidence of peach yellows in parts of orchards adjacent to woodland or other native vegetation than in the parts of orchards farther removed. He states that the trees immediately adjacent to the woodland were not necessarily the first to become diseased.

McCubbin (16) found from a study of orchards in Pennsylvania that the number of diseased peach trees in the outer rows was about 25 times the proportion found in interior trees. He made a study of a number of orchards where yellows trees were found in groups adjacent to areas of natural and permanent vegetation and cites a number of typical cases where the yellows group occurred near native vegetation. In one three-year-old orchard 26 diseased trees were found around a sink hole. Four additional trees became diseased the following year near this sink hole, all within 100 feet of its border.

From a list of 204 species of plants found growing adjacent to groups of peach trees affected with yellows McCubbin (16) found seven species common to six groups. An examination of this list shows that *Prunus avium* L., *P. serotina* Ehrh., and *P. virginiana* L. are listed in six out of nine groups studied. *Prunus americana*, however, is not listed. The writer has noted the association of the disease with the latter species in orchards in Pennsylvania that persistently showed a high incidence of disease in spite of inspection and careful removal of diseased peach trees. In one instance near Lebanon a group of five diseased trees were found within 160 feet of a clump of *Prunus americana*. The previous year the grower said that a diseased tree had been removed near the center of this group that doubtless served as the source of infection.

In July 1927 the writer observed a group of diseased peach trees on the periphery of a sink hole grown up to native vegetation (Fig. 5 E) in the center of a seven-year-old peach orchard containing 1900 trees, near

A



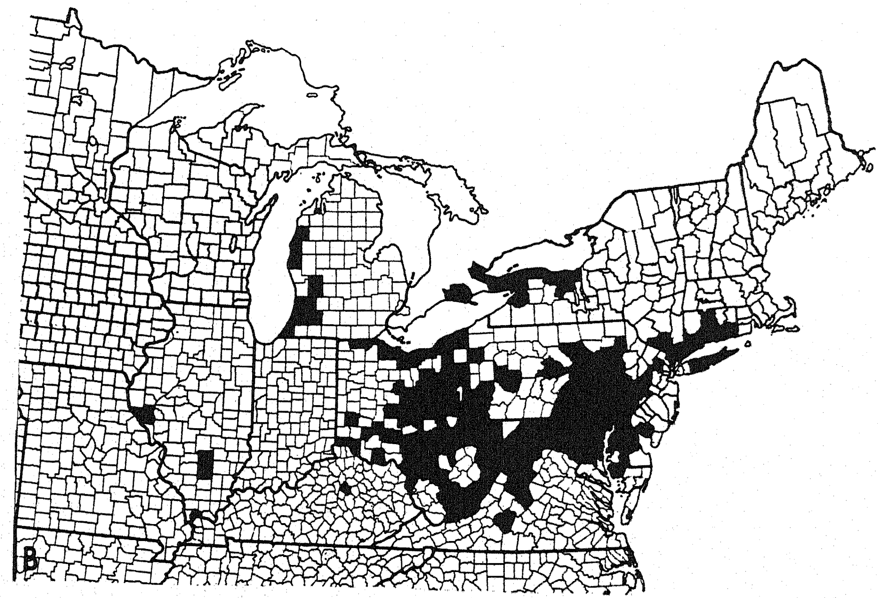


FIGURE 7. Distribution in the United States and Canada of: A. *Macropsis trimaculata* in relation to its host, *Prunus americana*, and the climatic range of the peach; and B. Peach yellows. Areas in solid black include counties known to be infested. The outer limit of peach yellows is not indicated on the map because it is not definitely known.

Cornwall, Pennsylvania. This was the only place in the orchard where the disease occurred.

The accompanying map (Fig. 7 A) gives the known distribution (8, 14, 23, 32, 34) of *Macropsis trimaculata*. It will be noted that it roughly corresponds with the known distribution of peach yellows (Fig. 7 B). Its southern range corresponds with the southern limit of peach yellows, although its principal host, *Prunus americana*, extends much farther southward (9, 11, 17).

It will be noted by a comparison of Figure 7 A and B that peach yellows is limited to the northern and eastern part of the climatic range of the peach in North America (10). Insofar as the writer is aware there are no authentic records of peach yellows in Georgia and California, which include the two most important peach growing districts in North America. Information received from State and Province Departments of Agriculture and other sources indicate that the range of the disease extends from northern Massachusetts westward through central New York, southern Ontario to western Michigan, southward to eastern Missouri, and eastward through Kentucky, Tennessee, Virginia, and the Carolinas. The disease is believed to be practically non-existent west of the Mississippi River.

The removal of wild plum from the vicinity of peach orchards combined with the general practice of roguing diseased trees would doubtless aid in the control of this disease.

SUMMARY

Peach yellows was transmitted experimentally by means of both nymphs and adults of the plum and peach leafhopper (*Macropsis trimaculata*) from diseased peach trees to 14 young healthy peach seedlings. About 16 per cent of the trees exposed to infected leafhoppers took the disease.

Forty-seven other species of insects and mites failed to transmit the disease.

The disease was transmitted also experimentally by budding. Healthy seedlings budded with positively diseased buds showed symptoms of the disease from one to two years in advance of trees budded with normal-appearing buds from diseased trees.

The disease was not transmitted by means of diseased pollen or by mechanical inoculation.

The habits of the insect vector differ markedly from that of most species of leafhoppers. This with the paucity of its population on peach has no doubt delayed its detection as the vector of peach yellows.

A study was made of the life history, habits, and population of *Macropsis trimaculata*.

A positive correlation of the insect vector to wild plum and the incidence of the disease was noted.

The removal of wild plum from the vicinity of peach orchards combined with the usual practice of roguing diseased trees are suggested as a possible means of control.

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SEVERAL CHEMICAL GROWTH SUBSTANCES WHICH CAUSE INITIATION OF ROOTS AND OTHER RESPONSES IN PLANTS[1]

P. W. ZIMMERMAN AND FRANK WILCOXON

In 1933 two papers published from this laboratory (15, 17) reported that carbon monoxide and three unsaturated hydrocarbon gases caused initiation and stimulation of adventitious roots on stems and leaves of several varieties of growing plants. The same chemicals caused epinasty of leaves, proliferations on stems, and anaesthesia of growing parts (2, 16).

Went (12) reported a test method for rhizocaline, a root-forming substance, whereby an approximation of the concentration could be determined by the number of roots that were formed on cuttings of sweet pea which had been treated with this material. Thimann and Went (10), using this assay method, determined the comparative amounts of this root-forming substance occurring naturally in plant and animal material. Laibach, Müller, and Schäfer (8), using concentrates from urine and pollen mixed with lanolin applied locally to plants, influenced callus formation, cell division, and increased root growth of cuttings. Kögl, Haagen-Smit, and Erxleben (7) were the first to isolate a growth substance ("hetero-auxine") as a pure chemical compound and identify it as indoleacetic acid (β-indolylacetic acid). Hitchcock (5) of this laboratory, using indoleacetic acid, indolepropionic acid, phenylacrylic acid, and phenylpropionic acid dissolved in water, oil, or in lanolin, and applied locally to intact plants, caused initiation of roots, epinasty of leaves, and bending and swelling of stems of several varieties of plants.

Within the last few months nine new chemical compounds have been found to have special formative effects on plants. They induce adventitious roots, epinasty of leaves, bending and swelling of stems, and anaesthesia. The purpose of this paper is to report the comparative effectiveness of these substances and the responses made by plants.

METHODS AND MATERIAL

The compounds were used as distilled water solutions or mixed with lanolin (U.S.P.). In a few cases where cuttings were involved, dilutions were made with Knop's solution. The cuttings were placed in vials or flasks so that the basal ends were immersed in the solutions. The water solutions were introduced into the stems and petioles of growing plants by means of glass tubes drawn to a capillary at one end. The capacity of the tubes varied but held on the average approximately 0.3 cc. of solution. The capillary end of the tube was inserted into the stem or petiole and left to drain into the plant. There was considerable variation in the length

[1] This article was preprinted July 24, 1935.

of time required for the tubes to empty. Also the response of the plant varied with the rate at which the substances drained from the tubes.

Lanolin preparations were applied locally by rubbing the mixture on stems or leaves with a glass rod. The exact amount of each compound used per gram of lanolin will be reported under the heading of results. The usual concentration range was from 0.01 per cent to 2 per cent.

The following species of plants were used in the experiments: African marigold (*Tagetes erecta* L.), tomato (*Lycopersicon esculentum* Mill.), buckwheat (*Fagopyrum esculentum* Moench.), sweet pea (*Lathyrus odoratus* L.), Windsor bean (*Vicia faba* L.), sunflower (*Helianthus debilis* Nutt.), Jerusalem artichoke (*Helianthus tuberosus* L.), dahlia (*Dahlia variabilis* Desf.), sensitive plant (*Mimosa pudica* L.), and *Chenopodium album* L.

SOURCE OF COMPOUNDS TESTED

Indole derivatives. A number of indole derivatives were obtained through the courtesy of Dr. R. H. Manske[2] of the National Research Council, Ottawa, Canada. Among them were included the following compounds: β-[2-carboxy-indolyl-(3)]-propionic acid, β-[2-carboxy-6-methoxy-indolyl-(3)]-propionic acid, β-[7-methoxy-indolyl-(3)]-propionic acid, γ-[indolyl-(3)]-butyric acid, β-[2-carboxy-7-methoxy-indolyl-(3)]-propionic acid, β-[5-methoxy-indolyl-(3)]-propionic acid, β-[2-carboxy-indolyl-(3)]-ethyl phenyl ether, indolyl-(3)-succinic acid, γ-[2-carboxy-indolyl-(3)]-butyric acid, β-[6-methoxy-indolyl-(3)]-propionic acid, β-[2-carboxy-5-methoxy-indolyl-(3)]-propionic acid.

Naphthalene derivatives. Alpha-naphthaleneacetic acid and β-naphthaleneacetic acid were prepared from the corresponding methylnaphthalenes by bromination, conversion to the nitriles, and subsequent hydrolysis of the latter as described by Mayer and Oppenheimer (9). The α-compound melted at 131° C. and the β-acid at 139° C., after purification by dissolving in alkali, boiling with Norite charcoal and reprecipitating with hydrochloric acid.

Anthraceneacetic acid, fluoreneacetic acid, and acenaphtheneacetic acid. These compounds were prepared by heating the hydrocarbons with chloroacetic acid (13). The yields were small, but could be improved by adding aluminum chloride to the melt. According to the authors cited above, acenaphthyl-(5)-acetic acid is formed in this reaction, while in the case of the anthracene and fluorene compounds, the location of the substituent group is unknown.

Phenylacetic acid and mandelic acid. Phenylacetic acid was obtained from the Eastman Kodak Company, while mandelic acid was prepared as described by Vanino (11, p. 589). The latter acid melted at 118° C.

[2] It is understood that Dr. Manske is in position to synthesize and supply a limited quantity of various indole derivatives to anyone interested in these compounds.

RESULTS

The following eight compounds caused unusual activity when applied to growing plants: α-naphthaleneacetic acid, β-naphthaleneacetic acid, acenaphtheneacetic acid, indolebutyric acid, phenylacetic acid, anthraceneacetic acid, α-naphthylacetonitrile, and fluoreneacetic acid.

The principal responses shown by treated plants were local initiation of roots on stems and leaves, local acceleration or retardation of growth causing swelling and bending of stems, epinasty, hyponasty or twisting of leaves according to the place the compounds were applied, and possibly local anaesthesia.

Initiation of roots. Applied to the stems, all of the compounds mentioned above definitely caused local initiation of roots on growing plants of tomato, sunflower, marigold, artichoke, buckwheat, dahlia, and tobacco. Alpha-naphthaleneacetic acid and indolebutyric acid were especially effective for initiating roots on both stems and leaves. If the plants were kept in a glass case after treatment, the new roots forced their way through the epidermis and out into the humid atmosphere (Figs. 1 and 2). The time required for roots to make their appearance varied with the chemical substances used, the concentration, the species of plants, and the exact place on the plant where the material was applied. Table I indicates the

TABLE I

EFFECTIVE CONCENTRATION RANGE OF FIVE GROWTH SUBSTANCES FOR TOMATO, EXPRESSED IN PER CENT IN LANOLIN

Chemical substances	Causing negative bending of stem or epinasty of leaves	Inducing adventitious roots
α-naphthaleneacetic acid	0.0100 – 2.0	0.1 – 2.0
Indolebutyric acid	0.0100 – 2.0	0.1 – 2.0
Indoleacetic acid	0.0003 – 2.0	0.4 – 2.0
Indolepropionic acid	0.0250 – 2.0	1.0 – 2.0
Phenylacetic acid	0.0250 – 3.0	1.0 – 3.0
Fluoreneacetic acid	0.0500 – 3.0	1.0 – 3.0

comparative effectiveness of several chemical compounds causing rooting, swelling, and negative (away from side where substance was applied) bending of tomato stems. Positive (toward side where substance was applied) bending occurred when the concentration of the substance was high enough to injure the tissue or retard the normal rate of growth. In general, it might be stated that the most effective concentration for root initiation falls just below that causing positive bending of the stem or at the point where there is slight retardation of growth. This, however, has not been definitely determined. When the concentration was high enough to cause evident injury, roots appeared on the opposite side, or adjoining the place on the stem where the substance was applied. This result indicates that

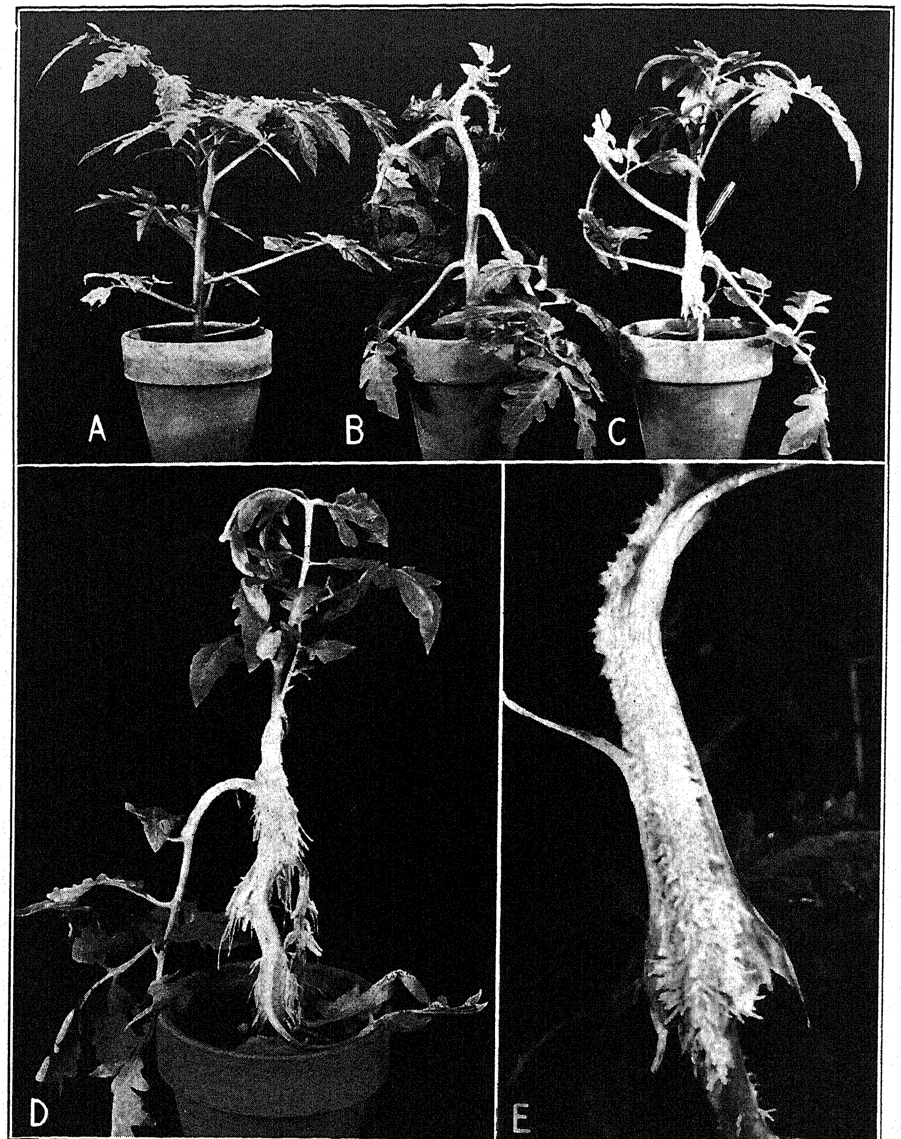


FIGURE 1. Tomato plants treated with growth substances. (A) Control; (B) Plant 24 hours after treatment along the stem with lanolin paste containing 1% α-naphthaleneacetic acid. (C) Approximately 0.4 cc. of 0.01% α-naphthaleneacetic acid in water injected by means of a glass tube caused local epinasty and initiation of roots; appearance after 8 days. (D) Stem and leaves of plant treated with lanolin paste containing 2.0% α-naphthaleneacetic acid. (E) Adventitious roots on stem 14 days after treatment with 2.0% indoleacetic acid.

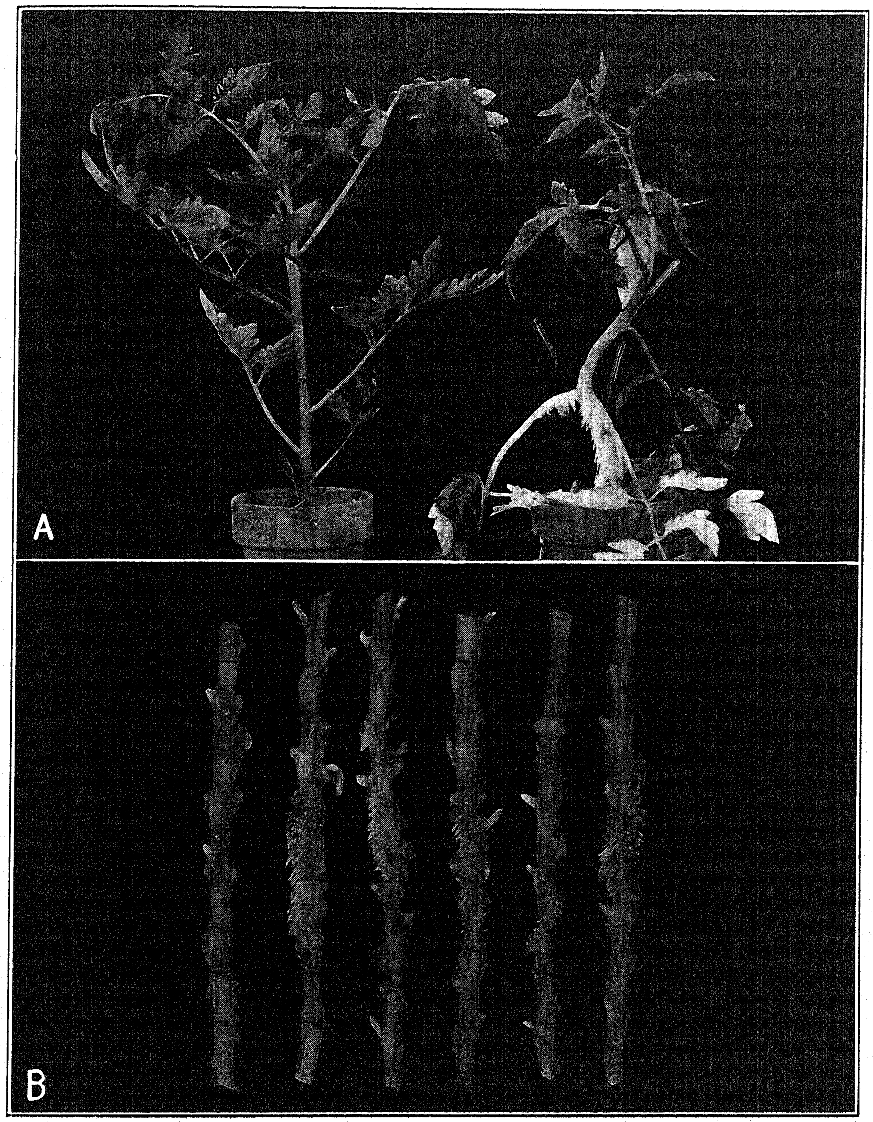


FIGURE 2. (A) Tomato plants. Left: control; right: 14 days after injections of 0.01% indolebutyric acid which induced epinasty of leaves and initiation of roots. (B) Adventitious roots on portions of tobacco stems cut from intact plants which had been treated over the region of elongation with lanolin preparations of growth substances. Left to right: control; 1.0% α-naphthaleneacetic acid; 1.0% indolebutyric acid; 0.4% indoleacetic acid; 0.5% indolepropionic acid; 2.0% phenylacetic acid.

as the substance diffused away from the point of toxic concentration, a range was reached which was effective for inducing roots. Under favorable conditions, 5 to 10 mg. of indolebutyric acid or α-naphthaleneacetic acid per gram of lanolin caused roots to be induced and appear through the epidermis on tomato in 6 days, marigold in 5 days, tobacco in 8 days, and artichoke in 6 days. Twenty milligrams of phenylacetic acid per gram of lanolin caused similar responses. One milligram of indolebutyric acid or naphthaleneacetic acid per gram of lanolin was effective but usually required two days more to induce roots.

Under comparable conditions similar concentrations of indoleacetic acid were not as effective for producing roots as either indolebutyric or α-naphthaleneacetic acid. Hitchcock (5) reported indolepropionic acid less effective than indoleacetic acid. All of these chemical substances, however, were very effective for inducing roots and when the most favorable concentration for each was used, there were only small differences in the final results. The indole and naphthalene compounds were effective over a much wider range than phenylacetic acid. The upper and lower limits for effectiveness of the different chemicals are shown in Table I. The upper limit refers to that concentration which does not appear to kill the tissue at the point of application; the lower limit refers to the lowest concentration which with one application induced rooting, though the roots might have been comparatively few and the time required longer than for higher concentrations. As a rule, roots appeared on treated stems two to five days earlier than on treated adjacent petioles. The older or middle-aged leaves of tomato produced roots more readily than young leaves. When tobacco plants 15 inches tall were treated from tip to base of the stems with effective concentrations of any of the five chemical substances mentioned above, roots formed most readily along the region of elongation extending from near the tip downward for a distance of two to three inches (Fig. 2B). This response resembles in some respects that caused by carbon monoxide and the three unsaturated hydrocarbon gases as reported from this laboratory in 1933 (15, 17). The tomato and marigold plants rooted wherever the growth substances were applied, even to the very tip. The youngest tissue, however, did not produce roots as readily as middle-aged or old tissue (Fig. 2A). This also resembles the response these plants made to the gas treatments.

Where a generous supply of lanolin containing two to three per cent α-naphthaleneacetic acid was applied to stems or leaves, abnormal roots were produced with an abundance of root hairs down to the tip. Also short, chubby roots often resulted from high concentrations of the substance (Fig. 1D). As a rule, roots induced with special substances had a white color and came out of the stem at an angle of approximately 45°, but α-naphthaleneacetic acid brought out a pink shading to brown color

of the roots, and they grew downward nearly paralleling the stem. These qualitative differences were in evidence whether the naphthalene compound was applied as a lanolin paste or injected as a water solution.

Water solutions of both indolebutyric acid and α-naphthaleneacetic acid were effective for producing roots over a range of 100 p.p.m. to 1 p.p.m. Neither the upper nor lower limits were definitely determined. Figures 1 and 2 show the response of tomato plants where the substances were injected with glass tubes drawn to a capillary at one end.

A convenient method for admitting water solutions of the substances directly into the vascular system is shown in Figure 4. The stem was slit upward with a knife so that the resulting overhanging stem piece could be immersed in the solution. By means of this method, upward translocation of the substances could be studied.

Ethylene as a root-forming substance. It was reported from this laboratory in 1933 that ethylene induced root initiation when intact growing plants were exposed to low concentrations of the gas (17). In the experiments at that time the entire plant was subjected to the gas and the effects were, of course, systemic. It now appears that local effects can be induced if the gas is dissolved in lanolin and applied as described for other growth substances.

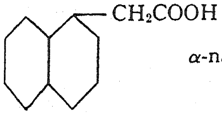
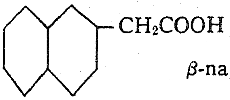
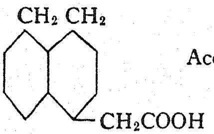
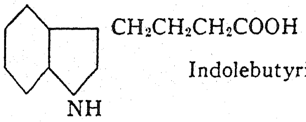
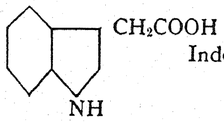
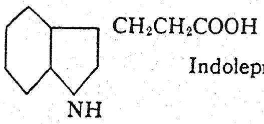
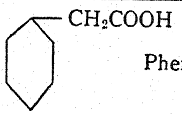
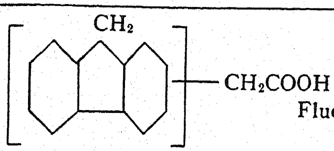
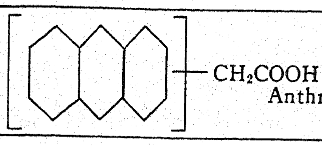
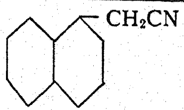
To obtain a solution of ethylene, the inside of a bottle was smeared with lanolin, after which the air was displaced with the gas and then the bottle stoppered and set in a cold place for several hours. The concentration of ethylene in solution was not determined because it could not remain constant when taken to a warm place where plants were treated. Since the gas escapes from lanolin more readily than the crystalline compounds, applications to the same region of the stems were made on three successive days. Also a larger quantity of the paste was necessary than for the other substances.

When applied as described above to the middle region of a tomato stem of a plant 8 to 10 inches in height, numerous roots were initiated locally and could be seen emerging through the epidermis in six days. This is the same length of time required for production of roots after treatment with the best crystalline growth substances.

Local acceleration or retardation of growth. Growth substances applied to one side of active shoots caused negative or positive bending according to the concentration of the chemical and the plant species. Positive bending was assumed to be due to retardation in normal growth rate or injury to the tissue where the chemical was applied. Negative bending was due to acceleration of the growth rate on the treated side of the stem. Figure 3 shows the result with artichoke plants of treating one side of the stems with concentrations which accelerate the growth rate. Whitening and swelling of the bark tissue followed within 48 hours after application of con-

TABLE II

EFFECTIVE CONCENTRATION RANGE OF GROWTH SUBSTANCES CAUSING BENDING OF SWEET PEA STEMS EXPRESSED IN PER CENT IN LANOLIN

Chemical substance	For positive bending or injury	For negative bending of stems
 α-naphthaleneacetic acid	1.5	0.05 – 1.0
 β-naphthaleneacetic acid	10.0	1.0 – 5.0
 Acenaphthyl-(5)-acetic acid	2.0	0.05 – 1.0
 Indolebutyric acid	2.0	0.025 – 1.0
 Indoleacetic acid	0.5	0.0005 – 0.4
 Indolepropionic acid	2.0	0.025 – 1.0
 Phenylacetic acid	3.0	0.25 – 2.0
 Fluoreneacetic acid	1.0	0.1 – 0.5
 Anthraceneacetic acid	—	— – 1.0
 α-naphthylacetonitrile	15.0	0.05 – 0.1

centrations which cause pronounced negative bending. Sweet pea seedlings an inch or more in height showed positive bending (toward the side of the stem where the substance was applied) when the stems were treated on one side with 1.5 per cent in lanolin of α-naphthaleneacetic or 2 per cent of indolebutyric acids. One per cent of these substances caused negative bending. Table II shows a range of effective concentrations for all the compounds tested.

Tomato stems showed negative bending with 2.0 per cent in lanolin of indoleacetic acid, indolepropionic acid, and phenylacetic acid, while the same concentrations of naphthaleneacetic acid and indolebutyric acid caused a positive response. It should be possible to determine intermediate concentrations in all cases which do not cause bending in either direction. For example, 0.5 per cent indoleacetic acid in lanolin, while increasing the diameter of the sweet pea and Windsor bean stem tips, did not cause bending. Slightly lower concentrations, however, caused negative bending.

Sweet pea and Windsor bean seedlings were retarded in elongation but increased in diameter of the stem tips by various concentrations of any of the growth substances (Table III, Fig. 3). A detailed microscopic study has not yet been completed but cross sections of fresh material observed with the aid of a microscope indicated that much of the swelling of the stems was due to the production of spongy, fluffy tissue outside the vascular cylinder. Retardation of Windsor bean treated on two sides with a series of concentrations was greatest with the strongest solution, decreasing with dilution down to a place where there was no apparent effect. Mention should here be made of the fact that Knight and Crocker (6) reported that ethylene caused retardation in elongation and swelling at the stem tips of etiolated sweet pea seedlings.

TABLE III

EFFECT OF INDOLEACETIC AND α-NAPHTHALENEACETIC ACID APPLIED IN LANOLIN TO OPPOSITE SIDES OF WINDSOR BEAN SEEDLINGS

Per cent of substance	No. plants measured	Av. height in cm. when treated April 12	Av. increase in height at three later dates		
			April 13	April 15	April 17
0.4 indoleacetic acid	10	12.1	2.9	4.8	8.3
0.1 indoleacetic acid	19	12.7	1.2	6.2	12.9
0.025 indoleacetic acid	18	12.5	2.3	8.7	17.5
1.0 α-naphthaleneacetic acid	9	16.2	2.2	6.0	10.0
Control	9	12.7	2.9	9.9	19.3
Control	9	13.4	2.6	10.0	20.6

Measurements for rate of growth of stems treated from the tip back to the base indicated first acceleration and then retardation of elongation (Table IV) even though the concentration was low enough to cause nega-

FIGURE 3. (A and B) Artichoke plants treated along one side of the stem with lanolin preparations of different substances. The upright plant in each pot is the control. Left to right the compounds are: 1.0% indolebutyric acid; 1.0% α-naphthaleneacetic acid; 1.0% indolepropionic acid; 0.4% indoleacetic acid. (C) Windsor bean seedlings treated along one side with 1.0% α-naphthaleneacetic acid in lanolin. Tall plants are controls. (D) Sweet pea seedlings. Same treatment as in "(C)."

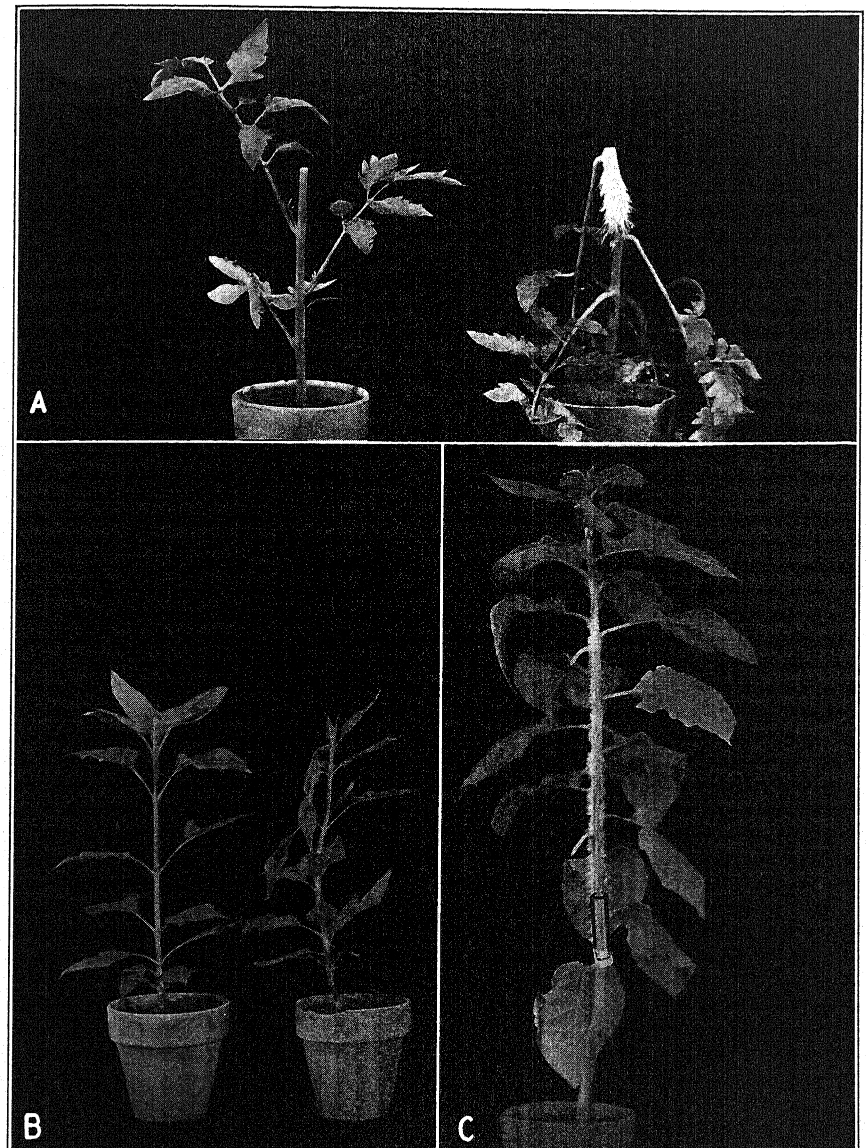


FIGURE 4. (A) Tomato plants. Left: control; right: cut surface treated with 1.0% α-naphthaleneacetic acid; photographed after 8 days. (B) Artichokes. Left: control; right: injected with 0.4 cc. of 0.5% solution of phenylacetic acid. (C) Tobacco with overhanging slit stem immersed in 0.05% indolebutyric acid. (Note roots and epinasty along path of translocation.)

TABLE IV

EFFECT OF A 0.5 PER CENT LANOLIN SOLUTION OF DIFFERENT COMPOUNDS APPLIED TO A TEN-INCH ZONE FROM THE TIP DOWNWARD

Compound	Plant	Height in cm. when treated May 1	Increase in height at four later dates			
			May 2	May 3	May 5	May 6
Lanolin control	Artichoke	31.0	1.0	4.0	6.5	7.5
α-naphthaleneacetic acid	"	32.0	3.5	6.0	6.5	7.5
"	"	28.5	3.5	5.5	5.5	6.5
Indolebutyric acid	"	28.5	3.0	4.5	5.5	6.5
Indoleacetic acid	"	28.0	4.5	7.0	7.0	8.5
Lanolin control	Tobacco	22.0	2.0	3.0	5.5	7.0
"	"	20.0	2.0	3.5	6.0	7.5
α-naphthaleneacetic acid	"	24.0	3.5	6.5	7.0	7.5
"	"	22.0	3.0	6.5	9.0	9.5
Phenylacetic acid	"	26.0	3.5	4.5	5.0	6.0
Indoleacetic acid	"	22.0	5.0	10.0	12.0	12.0
"	"	25.0	5.0	8.0	11.0	12.0

tive bending. To prevent bending so that measurements could be made, the lanolin containing the chemical was applied around the stem so the effect would be nearly the same throughout. Some bending occurred with this method, probably due to failure to make even distribution of the material.

Epinasty of leaves was induced by application of the substances in lanolin or by injecting the water solution by means of glass tubes. If applied in lanolin to a narrow zone around the stem of an active plant, leaves above and below this zone showed downward bending especially at the base of the petioles. Young leaves usually showed curling in addition to pronounced bending at the base. Epinasty of any single leaf was induced by applying the chemical lightly to a small region on the upper side of the petiole. The epinastic response of leaves to the growth substances was like that produced with carbon monoxide and unsaturated hydrocarbon gases (2, 16) except that when the plants were in an atmosphere containing gas the entire system was affected in contrast to local effects. Ethylene, propylene, and acetylene gas dissolved in lanolin or in water produced also the local effect when applied to the upper side of a tomato petiole. Some spreading occurred when enough lanolin containing the gas was used and if repeated applications were made, the entire plant responded as if it had been placed in an atmosphere containing gas. The result was similar to that described by Zimmerman, Hitchcock, and Crocker (18) showing that gas admitted to any part of a leaf spread throughout the entire system of the plant.

The likeness of the growth substances to the gases was further emphasized by the fact that the degree of epinastic response in both cases varied with the temperature. It was previously reported from this laboratory (14) that tomato plants did not make a typical response to ethylene gas

at 50° F. or less; above that temperature, however, the response increased with the temperature up to 73° F. Typical responses were best obtained when the temperature was 60° F. or above. This same variation in epinastic response held also for tomato plants treated with lanolin preparations containing 1.0 per cent of the growth substances.

When injected as a water solution into the stem of artichoke, indolebutyric acid was carried both up and down approximately eight inches from the point where it was admitted. The path of translocation could be seen by the epinastic response of the leaves (Fig. 4). Only leaves associated with the vascular bundles where the substance was injected showed epinasty. Alpha-naphthaleneacetic acid appeared to move upward a distance of 19 inches after an overhanging slit portion of an artichoke stem was immersed in a 0.5 per cent solution, as evidenced by epinasty of leaves along the stem to the tip of the plant. Here again leaves out of line with the vascular bundles of the overhanging stem piece did not respond to the chemical. Similarly, tobacco plants treated with indolebutyric acid appeared to carry the substance upward a distance of 16 inches. The path of translocation could be followed first by epinastic response of leaves and later by growth of roots initiated along the stem (Fig. 4). As can be seen in the picture, there were two distinct rows of roots on a line with the sides of the overhanging stem piece. There is evidence of lateral diffusion, since the stem below the place where the solution was admitted showed considerable root production. As the substance spread out of the main path of travel, some was carried downward, inducing roots along the way. Downward translocation was shown by applying the substances to the cut surface of a tomato stump (Fig. 4). The effect was evidenced by epinasty of leaves all along the stem and production of roots over a two-inch zone from the point where applied. This dual response indicates that a higher concentration is necessary for root initiation than for epinasty. The root response shown in Figure 4 illustrates also that the effect from the presence of a growth substance overcomes the influence of polarity, the roots in this case being produced at the tip instead of at the base.

Tomato cuttings five inches long with basal ends immersed in Knop's solution containing the effective substances showed declination of the uppermost leaves within a few hours' time. Hitchcock (5) reported this type of response for tomato cuttings in solutions of indolepropionic acid. The absolute lower limits for effective concentrations causing the response were not determined for the different substances. The following low concentrations, expressed in parts per million of water, were found to cause epinasty of tomato cuttings: phenylacetic acid 125, α-naphthaleneacetic acid 4, indolebutyric acid 20.

Hyponasty of individual leaves was induced by applying the substances to the under side of the petiole. Carbon monoxide gas caused epi-

nasty of all leaves, or what might be called a systemic effect. When the growth substance was applied to the under side of two petioles and then the entire plant subjected to an atmosphere containing one per cent carbon monoxide gas, two forces worked in opposition to each other. Figure 5 shows the appearance of tomato plants after such a treatment. It can be seen that the final result is not the typical effect from either the gas or the growth substance, but a combined effect. When a water solution of the growth substance was injected into the stem or leaf, epinasty resulted, indicating that the tissue on the upper side of the petiole is more susceptible than that of the lower side.

Response of Mimosa pudica to growth substances. Pulvinal cells of *Mimosa pudica* were rendered inactive when treated with lanolin containing one per cent of α-naphthaleneacetic, indolebutyric, or indoleacetic acid. At first it appeared as though the surrounding tissue had grown and thus clamped the pulvinal cells so they could not respond, but that assumption did not seem logical after the plant recovered and again made a normal response. Applied to the upper side of the pulvinal tissue, the petiole showed a slight declination before it became set or applied to the lower side, the petiole moved upward. In either case the tissue was rendered incapable of responding to external stimuli such as touch or sudden change in temperature. Figure 5 shows two pictures of a plant with the base of one petiole treated with α-naphthaleneacetic acid. The first shows the plant in a normal equilibrium position, the second after one leaf had been stimulated with the flame of a match. Note that the angle between the petiole of the treated leaf and the stem is practically the same in both pictures whereas other petioles moved downward. The condition of the treated pulvinal cells did not prevent the stimulus from passing through the petiole and into the leaflets which showed the normal response.

This condition suggests local anaesthesia. Previous reports from this laboratory concerned induced anaesthesia of *Mimosa* with carbon monoxide and unsaturated hydrocarbon gases (2, 16). When treated with those anaesthetics the entire plant lost its irritability, the gases causing a systemic effect. With a local application of the so-called "growth substance" the effect was local. The result may have been local anaesthesia.

Alpha-naphthylacetonitrile. The response of plants to treatment with this compound needs special mention. The substance is a product resulting from the reaction between sodium cyanide and naphtho-benzyl bromide, one of the steps in making α-naphthaleneacetic acid, which compound causes epinastic response of tomato leaves in less than two hours. However, this nitrile does not cause an evident response until the second day after its application, and then the response comes on slowly. It is effective when applied in lanolin or injected with the glass tube method with water. The final results were similar to those obtained with α-naphthaleneacetic

FIGURE 5. (A) Tomato plants given combined treatment of indoleacetic acid (0.4% in lanolin) and 1.0% carbon monoxide gas. Left: upper side of two middle leaves treated with the acid; right: lower side of two middle leaves treated with the acid. (B) *Mimosa pudica* 20 hours after base of one petiole (noted by arrow) had been treated with 1.0% α-naphthaleneacetic acid in lanolin. Left: appearance before flame test; right: one minute after flame of match was applied to tip of leaf on left. (Note change in position of leaves excepting one treated.)

acid. The type of response made by the plant suggests that the raw product, the nitrile, undergoes hydrolysis in the living tissue, forming an effective growth substance—possibly α-naphthaleneacetic acid.

Gaseous emanations from treated plants. Since the chemical compounds in question accelerate growth and since plant tissues are known to give off physiologically effective gases, the question arose as to whether treatment with these chemical substances would hasten the rate of metabolism and thereby increase the rate of production of emanations from the tissues. Three test plants, African marigold, *Chenopodium album*, and sunflower, previously reported as more sensitive to ethylene than tomato (2), were used in these experiments to detect gaseous emanations from plants. Treated and control tomato plants were inclosed with appropriate test plants in 18-liter bell jars. *Chenopodium album* inclosed with one 15-inch high tomato plant treated along the stem with 1.0 per cent indoleacetic acid in lanolin showed a pronounced epinastic response in 16 hours. Also the test plant inclosed with two large tomato plants showed a pronounced epinastic response. The test plants with the comparable control lots did not so respond in 72 hours. In one case where the test plant made a good response in 16 hours, a marigold plant was substituted for *Chenopodium*. Four hours later the marigold showed evident epinastic response, resembling plants treated with ethylene, propylene, acetylene, or carbon monoxide gas.

In the next set of experiments plants were treated with 0.2 per cent water solutions of indoleacetic acid injected along the stem by the glass tube method. Marigold plants were so sensitive to the emanations that they made a slight epinastic response with control plants. They made, however, a pronounced epinastic response where inclosed with treated plants. *Chenopodium* plants responded only where associated with treated plants. *Mimosa pudica* appeared normal with control plants, but where it was inclosed with two large treated tomato plants, the leaves of this sensitive plant lost their normal orientation to gravity, assumed a ruffled appearance, and lost their irritability to external stimuli. In every respect these plants appeared like those treated with carbon monoxide gas, where the response was described as anaesthesia (16). When inclosed with only one treated tomato plant, *Mimosa* plants became sluggish but had not entirely lost their irritability.

With proper selection of plants for variety, size, and activity, it should be possible to find types which as controls would not cause a response when inclosed with the most sensitive test plants. In this way the work could be standardized so that quantitative measurements could be made for the effectiveness of various concentrations of the different growth substances. Determinations are now being made of the change in respiratory rate due to acceleration of metabolism by means of growth substances.

When plants are treated with known concentrations of ethylene very definite formative effects resulted. Recounting some of them we find that ethylene induced epinasty of leaves, growth rigor, anaesthesia, and initiation of roots. Now that chemical compounds are found to hasten the production of ethylene or a like gas in plants, some of the effects attributed to so-called "growth substances" might be due indirectly to the unsaturated hydrocarbon gas produced in the tissue.

The conception that ethylene emanates from plants is well supported by previous published work. In 1933 Botjes (1) found by inclosing an apple with a tomato plant that the fruit gave off a physiologically effective gas which caused epinasty of leaves. Knowing how tomato plants respond to traces of ethylene in air, he concluded that ethylene emanated from the apples. Gane (4) went a step further by actually isolating ethylene from air surrounding apple tissue. Denny and Miller (3) recently have found that production of emanations causing epinasty of leaves is not restricted to fruit but they are given off also by various types of plant tissue including leaves, stems, and flowers. With such facts constantly increasing and with the actual identification by Gane of ethylene as the gas emanating from apple tissue, it appears as though ethylene might be associated in general with the normal metabolism of plants. If so, increase of the rate of metabolism would at the same time increase the rate of production of emanations from the tissue.

DISCUSSION

Previously eight chemical substances were known to induce initiation of roots: carbon monoxide, ethylene, propylene, acetylene, indolepropionic acid, phenylacrylic acid, and phenylpropionic acid reported from this laboratory, and indoleacetic acid isolated by Kögl and others (7). The present paper describes eight more chemical compounds which also induce initiation of adventitious roots. This makes a total of sixteen substances thus effective. Of the many thousands of known chemical compounds, there are probably many others which would be equally effective with those known to date. Considered from that angle, the recent disclosures militate against the idea of specificity for a particular "growth substance" or "hormone" unless we choose to put new meaning into those terms. It would seem more logical to speak simply of the response of plants to certain chemical compounds. If the plants manufacture their own growth-regulating substances, it is not likely that any one plant would make all of those known to be effective. Neither is it logical to assume that all plants naturally make and use one and the same growth substance. The delayed response of plants to α-naphthylacetonitrile reported under "results" suggests that the raw materials at hand may determine the kind of substances the tissues manufacture. Perhaps the growth of a given species

is not always regulated by the same substance but it may vary with the environmental conditions of the plant.

There was a qualitative difference in the response of plants to the different substances which deserves further consideration. Roots produced after treatment with indoleacetic acid were of a white color and grew away from the stem at an angle of approximately 45°. Alpha-naphthaleneacetic acid, especially in the higher concentrations, caused roots to develop a pinkish-brown color and grow downward, nearly paralleling the stem. Also, if the supply of the latter substance either in water or lanolin was maintained for five to ten days, some abnormally thick stubby roots appeared and the majority of roots were completely covered with root hairs. It has been shown (15, 17) that carbon monoxide and the unsaturated hydrocarbons change the orientation of roots and induce abnormal production of root hairs.

A characteristic response of sweet pea and Windsor bean seedlings treated with growth substances was abnormal enlargement of the stem tip (Fig. 3) and retardation in elongation. Growth is thus increased in one direction and retarded in another. From the standpoint of concentrations, indoleacetic acid was the most effective substance causing this response. As low as 0.0005 per cent was effective. The other substances caused the response, but with higher concentrations. In general, a concentration which caused bending of the stem also caused abnormal enlargement at the tip.

Through the kindness of Dr. R. H. Manske who supplied the material from his laboratory, it has been possible to test 12 new indole compounds for possible effectiveness as growth substances. The only active one of the list was indolebutyric acid, though, from the standpoint of molecular make-up, it is hard to believe that the slight difference in structure could account for the vast difference in effectiveness. As a root-forming substance, indolebutyric is more active than the other two effective indole compounds. As a growth-promoting substance for producing epinasty of leaves or negative bending of stems from local application, β-indolylacetic acid was the most effective and indolepropionic the least. In contrast with the highly active β-indolylacetic acid made by Dr. Manske, α-indolylacetic acid made in our laboratory was ineffective.

In the naphthalene series α- and β-naphthaleneacetic acid were both active but the alpha compound was approximately 100 times more effective than beta. There was a possibility of course, that the beta compound was slightly contaminated with the alpha. Compared with indole derivatives the α-naphthaleneacetic acid rates equally with indolebutyric for producing epinasty of tomato leaves and as a root-forming substance. Acenaphtheneacetic acid and β-naphthaleneacetic were much alike in effectiveness. With these two could be classed also anthraceneacetic acid

for mild activity. Fluoreneacetic acid falls somewhere between the last three named compounds and α-naphthaleneacetic acid from the standpoint both of root initiation and epinasty of leaves.

Leaves treated with high concentrations of the most effective substances made a very pronounced epinastic response from which they did not recover. If, on the other hand, the leaves responded to lower concentrations, recovery followed within a few hours or days, varying with the amount of material in solution available to the plant. This response indicates either that the plant utilizes the substances or that they deteriorate after entering the tissue. Water solutions of all the substances deteriorated on standing a few days; lanolin preparations, however, retained their effectiveness indefinitely. Leaves were slower to recover if a large amount of lanolin containing a low concentration of the substance was applied than where only a thin film was employed. In the latter case the entire supply probably entered the plant where it deteriorated or was consumed by the living tissue.

Ethylene, propylene, and acetylene dissolved in lanolin caused the same responses when applied locally as the lower concentrations of the other growth substances. Repeated applications of the ethylene or propylene preparations to the same region of tomato stems for three successive days also caused local initiation of roots.

SUMMARY

1. Sixteen growth substances have been considered as follows: β-indolylacetic acid isolated by Kögl and others and tested by Went and others; indolepropionic acid, phenylacrylic acid, and phenylpropionic acid reported by Hitchcock; carbon monoxide, ethylene, propylene, and acetylene reported from this laboratory as root-forming substances; and the following eight compounds reported in the present paper: α-naphthaleneacetic acid, β-naphthaleneacetic acid, acenaphthyl-(5)-acetic acid, indolebutyric acid, phenylacetic acid, fluoreneacetic acid, anthraceneacetic acid, and α-naphthylacetonitrile.

2. The principal plant responses induced by the growth substances were local initiation of adventitious roots on stems and leaves, proliferations, swelling and bending of stems, acceleration of growth, and epinasty of leaves.

3. Growth substances applied in lanolin paste caused local responses where applied though tending to become systemic when high concentrations were used. Water solutions of the substances caused systemic responses when the concentration was high, but local effects with lower concentrations.

4. The substances moved both upward and downward in stems. When injected by means of glass tubes drawn to a capillary at one end or ad-

mitted into an overhanging slit stem, the substances moved upward for a distance of 19 inches in the artichoke stem and 16 inches in the tobacco stem. The distance was indicated first by epinastic response of leaves and later by adventitious roots which were induced along the way.

5. All of the substances retarded elongation of sweet pea seedlings but increased the growth in diameter of the stem especially when applied at the tip. This response was like that induced by carbon monoxide gas and the unsaturated hydrocarbons.

6. There was some indication that the substances induced local anaesthesia in pulvinal cells of *Mimosa pudica.*

7. Alpha-naphthaleneacetic acid and indolebutyric acid are the most effective root-forming substances yet discovered; they are not as effective as indoleacetic acid for epinastic response of leaves. Ethylene or propylene dissolved in lanolin and applied locally for three successive days induced local initiation of roots in six days.

8. There was an indication that plants can use α-naphthylacetonitrile as raw material from which it can make an effective growth substance.

9. The production of emanations, thought to be ethylene, from growing plants was increased by treatment with growth substances. These chemical compounds accelerated growth, hastening metabolism, which in turn was thought to increase the production of ethylene by the living tissue. Increased growth resulting from the application of the special substances, as evidenced by the epinastic response, increased with the temperature, beginning at 50° F.

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SIMILARITIES IN THE EFFECTS OF ETHYLENE AND THE PLANT AUXINS[1]

William Crocker, A. E. Hitchcock, and P. W. Zimmerman

Similarities between the effects of ethylene and the plant auxins in modifying the growth of various plant organs and in initiating roots and intumescences are of great interest. The close agreement between the minimum effective concentration of ethylene and the auxins is likewise worthy of note. Finally, ethylene, like auxins A and B, is produced in plant tissues and may act as a natural developmental regulator in plants in a similar manner to the auxins.

Beginning with the striking experiments of Boysen-Jensen (3) on decapitated *Avena* coleoptiles, in which he showed that restoration of the removed tip restored the power to respond to light, the knowledge of the plant auxins has been developed mainly in the European laboratories and especially in the plant physiology laboratory and the organic chemistry institute of the University of Utrecht (30). We owe to F. W. Went and his father, F. A. F. C. Went (29), much of the knowledge and theory of the physiological action of the substances and to the former the excellent quantitative methods for studying the effects of the auxins. To Dr. Fritz Kögl (19) we owe the name auxin and the knowledge of the chemical constitution of the auxins. Beginning with the work of Neljubow (22, 23) on the effect of ethylene upon plants, the knowledge of the effect of ethylene upon plants has been developed in various American and European laboratories. Since the knowledge of the effects of these two groups of substances was developed in different laboratories little attention has been given to the similarity of their physiological effects. It is the purpose of this paper to compare the effects of these two groups of chemicals and certain other chemicals studied in this laboratory.

Our extensive studies of the effects of various gases upon plants (6, 33, 34, 35) show that three other gases (acetylene, propylene, and carbon monoxide) and possibly a fourth (butylene) have many if not all of the anaesthetic and stimulative effects upon plants that are shown by ethylene. While the effects of acetylene, propylene, and carbon monoxide on plants are similar to those of ethylene, the minimum required concentrations of these gases to produce such effects as petiole epinasty and declination of legume seedlings are much higher. Table I, taken from our previous article (6, p. 194) on ethylene-induced epinasty, shows the minimum concentration of these gases required for declination of the sweet pea seedling and for epinasty of the tomato petiole.

[1] This article was preprinted July 24, 1935.

TABLE I

COMPARATIVE EFFECTIVENESS OF GASES IN PRODUCING EPINASTY IN TOMATO PETIOLES AND DECLINATION IN SWEET PEA SEEDLINGS

Gas	Minimum parts per million needed to produce	
	Declination in sweet pea seedlings, according to Knight and Crocker*	Epinasty in tomato petiole**
Ethylene	0.2	0.1
Acetylene	250.0	50.0
Propylene	1000.0	50.0
Carbon monoxide	5000.0	500.0
Butylene	—	50,000.0

* 3 days' exposure used.
** 2 days' exposure used.

It will be noted that all of these gases are unsaturated, carbon-containing gases and that in the olefine series the minimum effective concentration increases rapidly as the number of carbon atoms in the chain increases. Ethylene is 500 to 1000 times as effective as propylene and propylene is about 1000 times as effective as butylene. Butylene proves so low in effectiveness that it has little interest as a growth modifier and exciter. Amongst the many other gases and vapors that were studied (6) and failed to give effects similar to ethylene were several unsaturated carbon compounds (allyl alcohol, acrolein, and isoprene) and all of the saturated carbon compounds tested. Later work has shown that butadiene ($CH_2=CH-CH=CH_2$) also is not effective in producing epinasty, although it has two points of unsaturation in the carbon chain. The four effective gases have three characteristics: they are carbon-containing, short-chained, and unsaturated. Kögl (19, p. 21) emphasized the fact that saturating the double bond or substituting another group for the carboxyl group in auxins A and B rendered them entirely ineffective. On this basis we thought perhaps that a carboxyl group on butylene, which is already unsaturated, would raise its epinastic-inducing power and other stimulative effects. Crotonic acid, the acid form of one of the homologs of butylene, however, proved entirely ineffective in inducing epinasty or other responses characteristic of the short-chain, unsaturated, carbon-containing gases. It seems evident that the carboxyl group in the short-chain gases does not have the significance shown in the auxins.

Before discussing in detail the various effects of ethylene upon plant development it is well to point out the general nature of its effects upon plants. What is said in this respect about ethylene applies also to acetylene, propylene, and carbon monoxide. These substances are preeminently anaesthetics and stimulants for plants and have extremely low toxicity, or tissue-killing properties. Ethylene has been applied to plants in various concentrations from 25 per cent of air to a fraction of a part to a million

of air for periods varying from hours to days without actually killing tissue and yet with anaesthetic and stimulative effects appearing in all the concentrations. Van der Laan (20, p. 736) of the Utrecht laboratory has compared the effects of ethylene and carbon monoxide upon plants to those of hydrocyanic acid. This is a very unhappy comparison, for hydrocyanic acid is a marked tissue-killer and the necessary increase in concentration to shift the effect from growth inhibition to extensive tissue-killing is slight. Whatever similarity of effect may exist between hydrocyanic acid and carbon monoxide upon mammals there is certainly no general similarity of effects upon plants. The use of ethylene as a general anaesthetic (21) in surgery with the absence of most, if not all, of the bad after-effects of ether and other general anaesthetics indicates that its effects on the mammal are hardly to be compared with those of the deadly hydrocyanic acid. Later in this article it will be seen that Van der Laan worked only with relatively high concentrations of ethylene, 5 to 50 p.p.m. of air, while the minimum effective concentration for reactions in some plants is 0.02 to 0.01 p.p.m. (27, p. 529). The high concentrations he used have very marked growth-inhibiting effects.

SPECIFIC EFFECTS

1. Epinasty of petioles. The epinasty of petioles is one of the more general responses of plants to ethylene and other unsaturated, short-chained, carbon-containing gases. Out of 202 different species and varieties of plants tested (6) 44 per cent gave epinastic reaction of the petioles to ethylene. The epinastic response of the leaves of the tomato (5, 6) and several other plants has been so thoroughly studied both as to the gases that induce the response and the concentrations of the several gases required to induce it that these plants can be used as reliable tests for the presence of ethylene. Hitchcock (16) has shown that indole-3-*n*-acetic acid, or hetero-auxin, indole-3-*n*-propionic, phenylacrylic, and phenylpropionic acids will all produce leaf epinasty when applied unilaterally to the upper side of the petiole or when applied as a solution equilaterally through the stem of the plant. It is difficult to apply ethylene and other effective gases in known concentrations otherwise than equilaterally (36) because they diffuse readily throughout the entire intercellular system of the plant. Besides hetero-auxin and the compounds mentioned by Hitchcock, Zimmerman and Wilcoxon (38) find that α-naphthaleneacetic, β-naphthaleneacetic, acenaphthyl-(5)-acetic acid, indolebutyric acid, phenylacetic acid, fluoreneacetic acid, anthraceneacetic acid, and α-naphthylacetonitrile are effective in inducing epinasty of leaves. They also find that placing the proper concentration of any of these compounds on any portion of the lower side of the petiole prevents the gas-induced epinasty in that portion of the petiole.

In so far as the unsaturated carbon gases cause the resumption of growth (6, p. 185–190) on the upper side of mature petioles it causes the elongation of plant cells. This is the original function discovered for the auxins and for a time the only function known for them. Indeed it is the very function that gave them their name.

2. *Intumescence formation.* Several workers (11, 14, 26, 27, 28) have shown that ethylene produces intumescences in many different plant tissues. These intumescences generally originate from phellogen of the cortex of stems or roots. Intumescences are also commonly induced in lenticels. The premature abscission of leaves caused by ethylene and other unsaturated gases also involves a marked enlargement and rounding up, if not a proliferation, of the cells of the abscission layer (37). According to Wallace (28) the ethylene-induced intumescences in the apple twig may originate from any living cells between the phellogen and the cambium. The thickenings of the walls are digested away irregularly and finally the middle lamella is dissolved. The cells then enlarge sometimes to giant size, reaching a diameter as great as 360μ. There is also a proliferation of cells by normal mitosis. Zimmerman and Wilcoxon (38) find intumescence formed as a result of the application of hetero-auxin and other chemicals that have like effects.

3. *Effects upon respiration.* Harvey (13) found that except for three-hour periods when there was an increase in CO_2 production, ethylene depressed the respiration of the sweet pea seedling. He used, however, relatively high concentrations of ethylene in the air, 2 p.p.m. Later in this paper we will show that 1 part of ethylene in 40 million of air gives a measurable reduction in growth of the sweet pea seedling. Harvey used, then, 80 times the minimum concentration necessary to reduce elongation rate. Harvey also used a mercury seal for his respiration chambers. Mercury vapors (32) may have modified the respiration rate. It seems likely if he had used much lower concentrations of ethylene he would have found increased respiration for long as well as short periods. Denny (7) found that ethylene in the air, ranging from 1 part per 1000 to 1 part per 1 million of air, increased the respiration of lemons. The increase in respiration ranged from 100 to 250 per cent, the intermediate concentration giving the greater increases. It is likely that ethylene like other common anaesthetics decreases respiration in high concentrations and increases it in low concentrations. There is some dispute as to whether the auxins modify respiration. Kögl (19, p. 23) seems to doubt whether purified auxin modifies the respiration rate. Bonner (1) has shown, however, that sections of *Avena* coleoptile show an increase in respiration after they have been immersed in a rather highly purified growth substance from *Rhizopus suinus*. In face of this disagreement as to the effect of auxins upon respiration there is no conclusive reason for thinking that they act differently

from other substances. This is especially true when one considers the many other physiological effects that they have similar to those of ethylene and other substances studied by Hitchcock (16), also Zimmerman and Wilcoxon (38). The effect of auxins upon respiration rate of plant organs will be known only when its effects are studied in a considerable range of concentrations.

4. *Anaesthetic and growth-inhibiting effects.* Apparently a reduction in the rate of elongation is a universal effect of ethylene and the other effective unsaturated, carbon-containing gases (17, 20, 22, 23, 34, 37) upon flowering plants, provided the proper ranges of concentrations are used. These reductions in rate of growth can be caused by a wide range of concentration without permanent injury to the tissues. The amount of reduction in growth slowly increases as the concentration of the gas increases. About 4 parts of ethylene per 1 million parts of air (18) will completely inhibit the elongation of the sweet pea seedling without causing any permanent tissue injury. While the seedling in this concentration ceases to elongate it increases in diameter near the tip forming an almost globular enlargement (18). Even 1 part of ethylene in 40 million of air, 1/160th the concentration necessary to completely stop elongation, will reduce the elongation rate by about 17 per cent. This power of ethylene to reduce elongation rate, or in higher concentrations to stop it completely, without permanently injuring the tissue is a manifestation of its high anaesthetic action and its low toxicity. It produces complete or partial growth (elongation) rigor depending upon the concentration used.

It has been shown by motion pictures (6, p. 190–192) that in 2 parts of ethylene per 1 million of air the growing tips of the stems of tomato and sunflower plants are thrown into complete movement rigor. The stems recover from this rigor and begin normal or even excessive growth movements soon after removal to air free from ethylene. At the same time that the stems were anaesthetized by the above concentrations of ethylene the petioles of the leaves, including the old mature ones, were stimulated to grow on the upper side and gave marked epinastic response. We have here the phenomenon of one part of the plant thrown into complete rigor by ethylene while at the same time another part of the plant is excited to new growth. Our experiences indicate that considerably lower concentrations of ethylene will throw the stems of the tomato and sunflower into movement rigor and at the same time produce the epinastic responses of the leaves.

Motion pictures of *Mimosa pudica* (34, p. 209) show that 0.25 of 1 per cent of carbon monoxide in the air breaks up the orderly arrangement of the leaves and leaflets and renders the plant incapable of reaction to heat and contact. When the plant is again removed to air the leaves and leaflets gradually take on their normal orderly arrangement and the plants recover their ability to respond to heat and contact. The data mentioned

above in this section show the great power of ethylene and other unsaturated carbon gases to inhibit elongation of plant stems without injuring the tissues. They also show the high effectiveness of these gases as plant anaesthetics and indicate that reduction in elongation rate or the complete stoppage of elongation is a partial or complete anaesthetic rigor.

Except for the elongation of the upper side of the petiole in gas-induced epinasty we have not found any cases where ethylene and the physiologically similar gases cause increased elongation of plant organs. It is possible that aside from this case of the petiole these gases do not cause such elongation. Denny and Miller (9) have found that plant tissues generally through metabolism produce ethylene or a gas or gases that act physiologically like ethylene. It is possible that any attempt to enclose plants within small chambers and bring the ethylene to the proper concentration to induce elongation may lead to too high a concentration because of the production of ethylene by the plants themselves. The possibility of ethylene acting as a general stimulant to organ elongation needs and is now receiving further study in this laboratory.

In the early studies of the auxins (30) they were supposed to have as their sole function organ elongation, hence the name. Later it has been found that they inhibit organ elongation (20, p. 701) as well. In fact, inhibition of elongation (4) is the very basis of explaining negative geotropic response of the root according to the auxin theory. So a very prominent physiological effect of the unsaturated, carbon-containing gases, namely, the reduction in the rate of elongation, is an effect of auxins upon certain plant organs and according to Zimmerman and Wilcoxon (38) this is an effect of hetero-auxin on all plant organs if it is used in sufficiently high concentration. The auxins then act both as growth promoters and growth inhibitors—they are growth regulators.

By the proper application of hetero-auxin and other similarly acting chemicals to the sweet pea and *Vicia faba* seedlings, Zimmerman and Wilcoxon (38) obtained in them reactions similar to the reactions caused by ethylene, inhibition of elongation, swelling and declination of the tip. By the proper application of hetero-auxin to the stem of *Mimosa pudica* they could also anaesthetize an adjoining leaf so it would not respond to heat or contact. In time the leaf recovered its power to respond. There are many similarities of effects between ethylene and the auxins in modifying growth and in producing anaesthesia in plants.

5. Hastening ripening. Ethylene is very effective in hastening the coloring of citrus fruits (8). This is a matter of decomposing chlorophyll. It has a like effect upon certain leaves (37, p. 465) and upon the leaf stalks of celery (15, 24). Ethylene also modifies the metabolism of plants (13) including the hastening of coloration and other ripening processes of fruits.

The effects of the plant auxins on coloring and ripening of fruits and

upon metabolism of plants have not yet been studied. Their study in this respect will be far more difficult than that of gases, for gases can be applied in the atmosphere and diffuse through (36) the intercellular system of plants readily while the auxins must be applied in solution or suspension and will move through the tissue much less readily.

6. Initiation of roots. Went (31) has called the root-forming hormone from leaves, germinating barley, pollen, etc., rhizocaline. Thimann and Went[2] (25) have tested the highly purified crystalline auxins A and B and hetero-auxin furnished by Kögl for their root-initiating ability. The crystalline auxin A had little root-inducing ability but it had also lost its auxin power. Crystalline auxin B and hetero-auxin showed high rhizocaline effect. The auxins are easily inactivated by oxidizing agents so Thimann and Went attempted to get rid of the auxin action while still retaining the rhizocaline action by treating auxin B and hetero-auxin with various oxidizing agents. They state their conclusions from these experiments as follows (25, p. 459): "The inactivation of the rootforming activity was thus different in auxin B from that in heteroauxin, which would seem to indicate that the rootforming activity is not due to one substance present as impurity in both preparations. However, the other alternative, that the root-producing and growth-promoting hormones are identical, is not supported by the wide variations in the ratio of the two activities. In this respect it seems especially significant that in the purification of the auxin the activity in root formation is reduced by from 10 to 50 times. It is at least safe to conclude that the 2 hormones are extremely closely related. It is hoped to obtain conclusive evidence as to their identity or difference at a later date." Of course it will always be of some significance to know whether auxin A and B and hetero-auxin have rhizocaline power, but a part of this significance has been removed by the fact that so many different substances have been found to have very high and almost identical rhizocaline power. Zimmerman, Crocker, and Hitchcock (33, 34, 35) have found this to be true of ethylene, propylene, acetylene, and carbon monoxide; Hitchcock (16) for synthetic hetero-auxin, indole-3-*n*-propionic acid, phenylacrylic acid, and phenylpropionic acid; and Zimmerman and Wilcoxon (38) for α-naphthaleneacetic acid, acenaphthyl-(5)-acetic acid, indolebutyric acid, phenylacetic acid, fluoreneacetic acid, anthraceneacetic acid, and α-naphthylacetonitrile.

The fact, that synthetic hetero-auxin (16) and the hetero-auxin extracted from urine (19) both have high rhizocaline or root initiation effects, indicates that hetero-auxin itself and not an impurity in it is a rhizocaline,

[2] After this article was in press the following article appeared:

F. W. WENT (Pasadena, Calif.). Hormones, involved in rootformation. The phenomenon of inhibition. 2. Internat. Bot. Congr. Amsterdam 1935. Proc. 2: 267–269. Went says, "One of the rootforming hormones (rhizocalines) is identical with auxin: auxin a, auxin b and hetero-auxin are equally effective."

for one would hardly expect the same effective impurity in the substance derived in the two very different ways.

A brief description of the methods of initiating rooting by these various compounds is in order. If intact plants (33, 34, 35) are exposed to air containing a proper concentration of ethylene, acetylene, propylene, or carbon monoxide for a few days and then removed to a moist chamber free from the gas, profuse rooting results. In some cases, as the tobacco and hydrangea, the rooting is limited to the region of the stem that was growing at the time of exposure. In other cases, such as the tomato and African marigold, the rooting is general over most of the stems and often occurs on the petioles and veins of the leaves. After the roots have been formed as a result of such a gas exposure a second exposure to the gas followed by a later period in a moist chamber will lead to profuse development of root hairs and to a change in the orientation of the root tip to gravity. While the gases initiate both roots and root hairs, they inhibit the growth of these organs; consequently a period of exposure to the gas is followed by a period in a moist chamber free from the effective gas to permit the growth of the organs. Went (31, p. 446) found that while rhizocaline initiates roots it inhibits their growth. Hetero-auxin and the other chemicals reported by Hitchcock (16) and Zimmerman and Wilcoxon (38) that are effective in root initiation are solids and must be applied in solution or in suspensions in lanolin, olive oil, etc. These workers found that these substances move most rapidly in plants in the basal direction but there is considerable movement in the apical direction also. They conclude that Went (31) has over-emphasized directional or polar movement. In practice the solids that cause root initiation are much more serviceable than the gases for the reason that they can be applied locally where rooting is desired while the gases diffuse readily all through the plant and may produce undesirable effects in other parts of the plants.

The root initiation by plant hormones and other chemicals is of special interest because it involves organization changes. Such of these substances as are found in plants in sufficient concentration may be considered organization hormones. Since ethylene is a product of plant metabolism (9) and especially since other hormone-like substances stimulate its production (38) within the plant, it may act as an organization hormone. Such an effect remains doubtful, however, especially for stems for two reasons: first, vegetative organs apparently produce less ethylene than fruits (9), and stems unlike fruits lack effective coverings for retaining the gas.

MINIMUM EFFECTIVE CONCENTRATIONS

It is rather difficult to compare the minimal effective concentration of ethylene since it must be applied as a gas with the minimum effective concentration of auxins which must be applied in solution. Such a comparison can be approximated, however. For such a comparison let us select

the most sensitive plant response known to date to the auxins on one hand and to ethylene on the other. Auxin A or B will produce 10° bending (19) in the decapitated coleoptile of *Avena* when a block of agar saturated with 1 part of auxin A or B in 50,000,000,000 parts of water is set on one side of the cut surface of the decapitated coleoptile. Wallace (27, p. 529) found that 1 part of ethylene in 100 million parts of air will induce intumescences in Transparent apple twigs. We will consider the experiments he ran at 20° C.; we will assume further that ethylene has the same solubility in the apple stem as it has in water and that the apparent specific gravity of the twigs is unity; and we know that in equilibrium there is 12.2 per cent the amount of ethylene dissolved in a given volume of water as appears in the same volume of air above it at 20° C. On the basis of the statements above we can calculate as follows the proportion of the weight of the apple twigs that was ethylene when Wallace used 1 part of ethylene to 100 million of air, the minimum intumescence inducing concentration:

$$\frac{1}{100{,}000{,}000} \times 12.2 \text{ cc. of } C_2H_4 \text{ per } 100 \text{ cc. of apple twig}$$

$$\frac{1}{10^7} \times 12.2 \times \frac{28}{22{,}400} = \text{grams of } C_2H_4 \text{ per } 100 \text{ grams of apple twig} =$$

$$\frac{1.52}{10^{11}} = \text{grams of } C_2H_4 \text{ in } 1 \text{ gram of twig, or}$$

1 gram of C_2H_4 per 658,000,000,000 grams of apple twigs.

On the basis of this calculation the apple twigs contain less than one-tenth the percentage by weight of ethylene with the minimum for intumescence formation as the water saturating the agar block contains of auxin A or B to give 10° bending of *Avena* coleoptile. On the per cent weight basis ethylene is more than ten times as effective in producing intumescences in apple twigs as auxin A or B is in inducing 10° bending in the oat coleoptile. Ethylene has a molecular weight of 28 while according to Kögl (19) auxin A has a molecular weight of about 328, or more than ten times that of ethylene. On the basis of molecular concentration ethylene seems to have about the same minimum effective concentration as auxins A and B. Such a comparison has its value merely in showing the same general magnitude of effectiveness. The auxins and ethylene are both effective in extremely high dilutions, a character commonly met in hormones and vitamins.

Because of past difficulties in getting large-sized cases that were gas-tight we redetermined the minimum concentration of ethylene necessary to produce epinasty in the leaves of tomato and the African marigold in two specially constructed cases of 543-liters capacity, one for the control and one for the treated plants. By use of these cases we also determined for the first time the minimum concentration of ethylene necessary to

produce epinasty in the leaf of the potato and the effect of various concentrations of ethylene upon the elongation rate of etiolated seedlings of sweet pea (*Lathyrus odoratus* L. var. Laddie) and *Lupinus* sp. (a tall annual variety furnished by Vaughan's Seed Store).

The front, both sides, and the top of each case were made of plate glass and the bottom and the back, which served as a door, were made of 16-gauge sheet steel. The glass plates and the bottom were fastened together with steel angle irons and the joints made tight by the use of rubber gaskets and white lead. The back, or door, was fastened on with 15 bolts projecting from the steel-grooved frame at the back. The bottom of the groove was provided with a fiber gasket against which a flange on the door was screwed for the seal. Before using the cases all joints were resealed with modelling clay which we have found a perfect seal against ethylene leakage. Also after putting the plants in the cases and before placing the door in position the groove in which the door flange fitted was filled with modelling clay. The door was then put into position and screwed tight by means of 15 large milled nuts. The door of the case was provided with tubes through which the gas could be forced. The experiments were run in darkness at 25° C. The duration of each experiment was two days. At the end of the first day the doors were removed from the cases and the room and cases thoroughly aired out by means of a large fan which gave a complete change of air in the room several times a minute. After this the doors were again screwed on and the gas run in.

In determining the epinastic response of the leaves the angle that each petiole formed with the stem of the plant was determined for both the check and treated plants before placing in the case at the end of a day and at the close of the experiment. In the check plants some leaves showed several degrees of epinasty, most leaves showed little or no epinasty, while occasionally a leaf showed slight hyponasty. In determining the degrees of epinasty shown by the leaves of the treated plants the degrees of epinasty shown by the checks were subtracted from that of the treated plants and no difference was considered significant unless it amounted to more than 10°. The concentrations of ethylene used in these experiments were 1:1 million, 1:10 million, 1:20 million, 1:40 million, 1:60 million, 1:80 million, and 1:100 million of air. No intermediate concentrations were tried. The following are the minimum concentrations of ethylene producing epinasty and the number of degrees of epinasty produced.

Plant	Minimum effective ethylene concentration	Degrees of epinasty produced
Tomato	1 part per 10 million of air	17
Potato	1 part per 40 million of air	18
African marigold	1 part per 60 million of air	15

While the tomato is the plant commonly used to detect ethylene in the air it is only one-fourth as sensitive as the potato and one-sixth as sensitive as the African marigold as determined by the minimum concentration necessary to produce epinasty. The tomato, however, has certain advantages for this purpose. It can be grown at any time, even during the shortest days of winter. It stands darkness and other bad conditions well during the two or more days of exposure. All the leaves respond including the oldest mature leaves. The amount of epinasty increases with the concentration of the ethylene until in sufficient concentration the petioles are all turned down nearly parallel with the stems. Potatoes are more difficult to grow and suffer somewhat even with two days' exposure to darkness. The old leaves begin to turn yellow under this condition even in air and show marked yellowing and fall of the old leaves if there is as little ethylene present as 1 part in 10 million. The African marigold is in most respects as desirable as the tomato and has the added virtue of responding to one-sixth the concentration of ethylene. We have found a number of other plants very sensitive to ethylene-induced epinasty but as yet have not worked out the minimum effective concentrations. Among these are sunflower (*Helianthus annuus* L.), buckwheat (*Fagopyrum esculentum* Moench.), *Chenopodium album* L., *Galinsoga parviflora* Cav. It may be that some of these will give epinastic response to ethylene in as low concentration as the apple twig gives the intumescence response.

For determining the effect of ethylene upon the elongation of etiolated seedlings of sweet pea and *Lupinus* the seedlings were grown in soil in pots in darkness. Five pots with about 24 seedlings in each pot were used for treatment and the same number for controls. As soon as the seedlings were far enough above the ground for measurement the height of each seedling from ground level to the tip was taken and the seedlings immediately sealed into the cases. The duration of the experiment was two days and the gas was changed at the end of the first day as stated above. Table II shows the effect of various concentrations of ethylene upon the elongation of these seedlings. The growth of the controls is considered as 100 per cent and the growth of the treated seedlings is reported in per cent deviation from the controls. The data for any single run under the conditions mentioned above have been subjected to statistical analysis by Dr. William J. Youden of this laboratory. This analysis shows that any deviation from the checks to be significant must be greater than 10 per cent. It is evident from Table II that 1 part of ethylene in 40 million of air still reduces the elongation rate of sweet pea seedlings and it is possible that even 1 part in 60 million has such an effect. It is also evident that 1 part of ethylene in 80 million of air reduces the elongation of *Lupinus* seedlings with the possibility that 1 part in 100 million has a like effect. Van der Laan (20) used 5 to 50 parts of ethylene per million of air in his

TABLE II

EFFECT OF ETHYLENE UPON ELONGATION OF ETIOLATED SEEDLINGS OF SWEET PEA AND LUPINUS. ELONGATION OF CONTROLS CALCULATED AS 100 PER CENT

Conc. of ethylene used	Per cent deviation from controls	
	Elongation of sweet pea	Elongation of *Lupinus*
1:10 million of air	−48	−30
1:20 " " "	−14	−16
1:40 " " "	−17	−25
1:60 " " "	−10	−20
1:80 " " "	−3	−17
1:100 " " "	−6	−12
1:120 " " "	+1	−1
1:140 " " "	−5	−1
1:160 " " "	−4	0
1:180 " " "	−6	−1
1:300 " " "	0	−8
1:500 " " "	+9	0
1:1000 " " "	+9	+5
1:2000 " " "	−1	+3

etiolated seedling experiments. Such concentrations are certainly highly depressant.

In the experiments just described the plants and seedlings were exposed to ethylene as they grew in the soil in pots with no attempt to cover the soil or pots to avoid adsorption of ethylene by them. If there was a differential adsorption of ethylene by the soil and pots the minimal effective concentrations given above are all too high. On the other hand, the fact that such high dilutions of ethylene gave responses indicates that differential adsorption did not play any considerable part for in high dilutions the adsorption ought to be nearly complete. There is also the possibility of the chemical union of ethylene with some soil or pot constituent. This, too, would remove gas from the air and make the minimum effective concentrations reported above too high. The fact that such high dilutions of ethylene gave responses tends to minimize the significance of this factor. If ethylene is produced by the plants themselves it would tend to make the minimum effective concentrations reported above too low. This ethylene would originate within the plant and be at the point of greatest effectiveness. The large volume of the cases in proportion to the volume of plants, also the readiness with which ethylene diffuses into, through, and out of plants, would, however, tend to minimize the significance of this factor.

From the data and discussion given in this section of the paper it is evident that the minimum molecular concentration of ethylene required to produce various physiological effects in plants, namely, intumescence formation, epinasty of leaves, and reduction in rate of elongation of etiolated seedlings, is approximately the same as the minimum molecular

concentration of auxins A and B required for producing the bending in *Avena* coleoptile and somewhat lower than the minimum molecular concentration of hetero-auxin (19) required for the bending of the *Avena* coleoptile. The minimum concentration of ethylene and of the auxins required for the effects mentioned above is extremely low. In respect to the low minimal effective concentration these substances resemble hormones and vitamins.

ETHYLENE AS A HORMONE

We may now turn to the third point mentioned in the introductory paragraph of this article, namely, the possibility that ethylene functions in plants as a hormone. We have already mentioned in this paper the many growth-modifying and organization changes caused in plants by ethylene, also the marked effect of ethylene in coloring or maturing fruits and other plant parts. Harvey (13) found ethylene to be very effective in producing changes in the general processes of plant metabolism giving a great increase in soluble sugars, amino acids, etc., at the expense of the insoluble carbohydrates, proteins, and fats. Recently, evidence has been accumulating that ethylene is a product of plant metabolism. Elmer (11) found that emanations from apples stunted the growth of potatoes. Botjes (2) found that the emanations from apples caused epinasty in tomato leaves and the triple response (18) of the pea seedling and that the effective portion of the emanations from apples was absorbed by an ethylene absorbent. Gane (12) proved by chemical methods that apples give off ethylene during storage. Denny and Miller (9) showed that not only fruits but also flowers and even vegetative parts such as leaves and stems give off emanations that cause leaf epinasty identical with that caused by ethylene. Some of the fruits were found to produce comparatively large amounts of the gas. Nelson and Harvey (24) have very recently shown that blanching celery gives off a gas that produces leaf epinasty. Finally, Zimmerman and Wilcoxon (38) found that treating tomato plants with hetero-auxin and several other chemicals that have similar physiological effects increases greatly the production of epinastic-inducing gases by the tomato plants.

The facts stated in this section leave little doubt that ethylene is produced by some fruits in sufficient amounts to further ripening. It is not unlikely that other metabolic products in fruits have similar effects. The well developed rind of fruits prevent the rapid escape of gases. The second and third sections of this paper show that ethylene has many growth-modifying and growth-initiating effects upon plants. Whether it is produced by the vegetative parts of plants in sufficient amounts and, if so, whether it is held within the plant sufficiently to enable it to act as a growth-modifying and organization substance or hormone must be answered by future research.

DISCUSSION

Van der Laan's article (20) on the effect of ethylene upon plants as explained by its effects upon auxin production merits special consideration. He found that the concentrations of ethylene he used reduced the elongation of etiolated seedlings very markedly and at the same time it almost completely stopped auxin production by the seedlings. He concludes that reduced auxin production accounts for the reduced rate of elongation. The high concentrations of ethylene Van der Laan used may depress many physiological processes within the seedlings besides auxin production and this general depressant effect rather than the specific effects on auxins may account for the reduced rate of elongation.

He made no experiments upon the mechanism by which ethylene induces leaf epinasty but ventured an explanation upon the assumption that ethylene reduces auxin production in the petioles as it does in seedlings. He speaks of leaf epinasty as autonomic nutation which under ordinary growth conditions is held in check by negative geotropism. With reduction in auxin the negative geotropism is weakened and epinasty results. Besides lacking any experimental basis this theory does not agree with two sets of facts that are now well established. Ethylene induces epinasty in mature petioles that have ceased to grow. In such petioles it is a matter of inducing new growth. This new growth, according to the hypothesis, is connected with lack of auxin or there is a negative relation between auxin and growth. Hitchcock (16) and Zimmerman and Wilcoxon (38) have shown that when hetero-auxin or many other physiologically similarly acting substances are applied to the upper side of the petiole or when applied equilaterally to the petiole through the stem produce epinasty just as does ethylene. The hypothesis assumes that ethylene causes epinasty by reducing the amount of auxin and yet these later experiments show that increased hetero-auxin in the petiole causes epinasty. Zimmerman and Wilcoxon have also shown that applying hetero-auxin to the lower side of the basal portion of the petiole will prevent CO-induced epinasty in that part of the petiole while the CO-induced epinasty occurs in the more distal part of the petiole beyond the region of action of the hetero-auxin. All of these facts indicate that the unsaturated, carbon-containing gases and hetero-auxin are identical in their power to induce leaf epinasty, which is quite contrary to Van der Laan's hypothesis.

Van der Laan emphasizes the high effectiveness of ethylene in furthering the ripening of fruits which he considers enzymatic processes. Much work confirms his view in this matter and later work shows further that ethylene is probably produced in fruits in sufficient quantities to act as a ripening hormone.

Van der Laan fails even to mention two of the most interesting effects of ethylene upon plants, namely, intumescence formation and initiation

of roots. The first effect has been known for years (10, 14, 26, 27, 28) and root initiation effects (33, 34, 35) were published one year before Van der Laan's article. Root initiation is an important organization effect; such a function as one might expect hormones to perform. The four carbon-containing, short-chained, unsaturated gases, ethylene, acetylene, propylene, and carbon monoxide, are practically identical in their root initiation power with hetero-auxin and various other similarly acting chemicals (16, 38). Here again the similarities of effects of the hetero-auxin and the unsaturated gases and not the differences are the striking thing.

From the facts stated in this paper it is evident that there are a number of organic chemicals that have similar growth-modifying and organization effects upon plants. The ones we know to date vary from a two-atom molecule, CO, with a molecular weight of 28 through the six-atom molecule, ethylene, with a molecular weight of 28, indoleacetic acid, or hetero-auxin, with a nitrogen ring and a molecular weight of 175, auxin B without nitrogen but with a molecular weight of 310, and, finally, possibly through auxin A with a molecular weight of 328. There is no common structural character in these compounds that will explain their similar physiological effects. Their physiological effects will likely finally be explained by changes that they bring about within the plants. Such an explanation must await the accumulation of many more facts. In fact the field of chemical control of plant growth and development needs at present the accumulation of many more facts rather than attempted theories to explain the facts.

Hormonal studies in plants will no doubt be benefited by a broad survey of the field of organic chemicals and their effects upon plant development. The field of endocrine studies in animals might also be greatly benefited by increasing the number of chemicals to be studied to include many compounds not known to be found in the animal. Working with pure known chemical compounds would tend to remove much of the mysticism from the endocrine field and, should known or easily synthesized chemicals prove effective in controlling various metabolic and developmental processes in the animal, they could be obtained in unlimited quantities at reasonable prices.

SUMMARY

1. The paper describes the effects of ethylene, also acetylene, propylene, and carbon monoxide, upon the growth and course of development of plants. It is found that these gases are preeminently anaesthetics and stimulants for plants and are low in toxicity or tissue-killing power. Their effects on plants are in marked contrast to those of hydrocyanic acid which burns plant tissues.

2. These gases produce leaf epinasty as do indoleacetic acid, or hetero-auxin, indolepropionic acid, phenylacrylic acid, and phenylpropion-

ic acid, studied by Hitchcock, and α-naphthaleneacetic acid, β-naphthaleneacetic acid, acenaphthyl-(5)-acetic acid, indolebutyric acid, fluoreneacetic acid, anthraceneacetic acid, and α-naphthylacetonitrile, recently studied by Zimmerman and Wilcoxon.

3. These gases induce intumescence formation in plants as do hetero-auxin and the other compounds mentioned under "2".

4. Ethylene apparently depresses or increases respiration rate, depending upon the concentration in the air surrounding the plant and upon the time and plant organ used. Whether auxins modify respiration rate or have no effect on it is still in question. More measurements are needed to settle this point.

5. Ethylene, acetylene, propylene, and carbon monoxide, depending upon the concentration used, reduce elongation rate or completely stop elongation without killing tissues. In producing epinasty of mature leaves they induce elongation of cells on the upper side of petioles. The auxins induce elongation of plant cells or inhibit elongation, depending upon the concentration used and plant organ studied.

6. Ethylene hastens the decomposition of chlorophyll and the coloring and other ripening processes of fruits. Auxins have not been studied in this respect.

7. Ethylene, acetylene, propylene, and carbon monoxide initiate root development. Hetero-auxin and the three other compounds studied by Hitchcock and the eight other compounds studied by Zimmerman and Wilcoxon have similar root-initiating power.

8. Ethylene will induce intumescence formation in Transparent apple twigs when it equals 1:658,000,000,000 parts of the weight of the twigs. Auxin A or B will induce bending in *Avena* coleoptile in 1:50,000,000,000 parts of water. Ethylene has less than one-tenth the molecular weight of auxin A or B, so the minimum effective molecular concentrations of ethylene on one hand and the auxins on the other are approximately the same. Both groups of substances are effective in extremely low concentrations, a common character of hormones and vitamins.

9. Ethylene is a product of the metabolism of plant organs, both vegetative and reproductive. There is much evidence that it acts as a fruit-ripening hormone in plants and a possibility that it acts as a growth-rate and organization hormone in vegetative organs of plants.

10. It is suggested that the knowledge of plant hormones will be much advanced by a study of the effect on plant development of a broad range of organic chemicals as exemplified by the study of the effects of the unsaturated, short-chained, carbon-containing gases and of the several compounds reported by Hitchcock and by Zimmerman and Wilcoxon. It is also suggested that progress in knowledge of endocrines in animals may be furthered by a similar broadening of the studies in that field.

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SEA-WATER TOLERANCE OF RUPPIA MARITIMA L.

W. S. Bourn

INTRODUCTION

Previous papers (3, 4) reported the results of experiments on the sea-water tolerance of six species of aquatic angiosperms, species which, although tolerant of certain amounts of salt water, occur naturally submerged in fresh water. The present paper deals with the results of experiments on the sea-water tolerance of *Ruppia maritima* L. (widgeongrass), one of the few angiosperms which, as Arber (2, p. 134) states, "typically occur in a brackish medium."

Ruppia maritima L. is widely distributed throughout the world. On the Atlantic Coast of North America it occurs from the maritime provinces of Canada to Central America. It is often found growing in dense mats in brackish ponds and on mud flats of coastal bays and estuaries. It is also found abundant in interior alkaline lakes and along the Pacific Coast. Sometimes it is found, as McAtee (10, p. 18) states, "in water that passes for fresh, as in the upper part of Currituck Sound, North Carolina, but inlets from the ocean to this part of the Sound have existed in recent years and high tides at times cross the narrow beach."

Being so widespread in distribution and having numerous nutritive seeds, luxuriant tender foliage, and succulent roots, *Ruppia maritima* L. has long been known (10) as one of our most important food plants for migratory waterfowl and is eagerly sought after by many species of wild ducks and geese. In the more brackish coastal waters of this country *Zostera marina* L. (eelgrass) in the past has been its only rival in superiority and in economic importance as a food plant for wildfowl. In recent years, however, *Zostera marina* L. has almost completely disappeared from the Atlantic Coasts of North America (6, 7) and *Ruppia maritima* L. now remains as practically the only duckfood plant of any appreciable importance in our brackish coastal waters. The natural habitat of *Zostera marina* L., however, has been a more saline medium than that believed to be tolerated by *Ruppia maritima* L. (6). Nevertheless, Cottam (7, p. 5), writing on the present situation regarding eelgrass along the coast of North Carolina, says, "Wigeongrass (*Ruppia maritima* L.) has spread considerably there during the past 4 years and now occupies much of the space formerly covered by the *Zostera*." Previously, Cottam (6, p. 193) had suggested that in the less salty waters where *Zostera* (eelgrass) had grown *Ruppia maritima* L. "might be used to make good the food and cover lost to waterfowl in the eelgrass catastrophe." It is of great interest, therefore, to determine under controlled conditions the concentration of sea water which *Ruppia maritima* L. will tolerate. The results of such a determination should be

highly valuable as information not only to conservationists and sportsmen but also to the fisheries industry as well.

EXPERIMENTAL METHODS

The experiments were performed in the greenhouse from March 1 to June 1, a season of the year in Yonkers when light conditions are most favorable for the growth of aquatic plants. Stoneware jars, 45 centimeters in depth and approximately 45 liters in capacity, were used as culture vessels. Ten kilograms of good soil were added to each vessel. This soil was composed of sod soil composted with one-tenth shredded manure and one-sixth humus; lime was added to bring the reaction of the composted soil to approximate neutrality. Sea water from Long Island Sound was used in the preparation of the culture solutions. This water was found, according to the field method described by Denny (8) for determining the saltiness of brackish water, to be approximately 80 per cent normal sea water, as a total salt concentration of 3.5 per cent is considered (5) normal sea water, although at times the concentration of the Sound water was determined to be as low as 70 per cent of the amount normally found in the ocean. The water was filtered, tested for salt content, and then diluted with tap water, or evaporated down, as the particular cultures required, to make the following concentrations of standard sea water: 0 (tap water), 5, 10, 15, 20, 40, 60, 80, 85, 90, 95, 100, 110, 125, and 150 per cent. The solutions thus made up were renewed as often as necessary to keep down the appearance in the cultures of algal growths. Frequent renewal of the solutions also aided materially in keeping down the temperature of the cultures, which fluctuated during the course of the experiments from 20° to 26° C. The approximate concentrations of dissolved carbon dioxide and oxygen found in the type of sea water used (5), namely, 40 and 6 parts per million by weight, respectively, were maintained fairly constant in each culture throughout the experiments. This was accomplished by bubbling the pure gases (carbon dioxide was obtained from the process of fermentation) from pressure cylinders into the culture solutions through Berkefeld filter cores. These clay filter cores caused the gases to be liberated uniformly, in the form of innumerable small bubbles, at the bottom of the culture vessels. The gas concentrations were then checked by the methods outlined by the American Public Health Association (1). It was found very necessary to add these gases to those solutions containing 80 per cent and more sea water, for in the preparation of such solutions evaporation to the salt concentrations desired practically depleted the water of both gases. To the cultures containing 60 per cent and less sea water, however, the addition of carbon dioxide alone was found necessary after the plants began to grow, for the growing plants, as shown before (3, 4), proved to be more efficient oxygenators than the artificial bubbling system.

The plant material used was taken from cultures grown for several months in the same greenhouse as that in which the present experiments were carried out. The stock was collected originally from Pocha Pond, on the island of Martha's Vineyard, Massachusetts, in water containing approximately 45 per cent sea water. For several months prior to the beginning of the experiments, the stock plants had been grown successfully in soil and culture solutions containing 20 per cent sea water. Ten terminal, unbranched cuttings, each 15 cm. in length and approximately equal in weight, were employed in each culture. These cuttings were carefully wiped, weighed, and then placed in solutions of 20 per cent sea water for two weeks to allow the formation of roots before transferring the plants to the experimental solutions. At the conclusion of the experiments the plants were carefully removed from the cultures, washed several times, and brought to a constant dry weight in an oven at 100° C. The percentage increase in dry weight in each culture was then calculated by comparing with the dry weights of comparable samples taken at the beginning of the experiments.

RESULTS

The effects of different concentrations of sea water on the growth of *Ruppia maritima* L. under favorable conditions of light, soil, temperature,

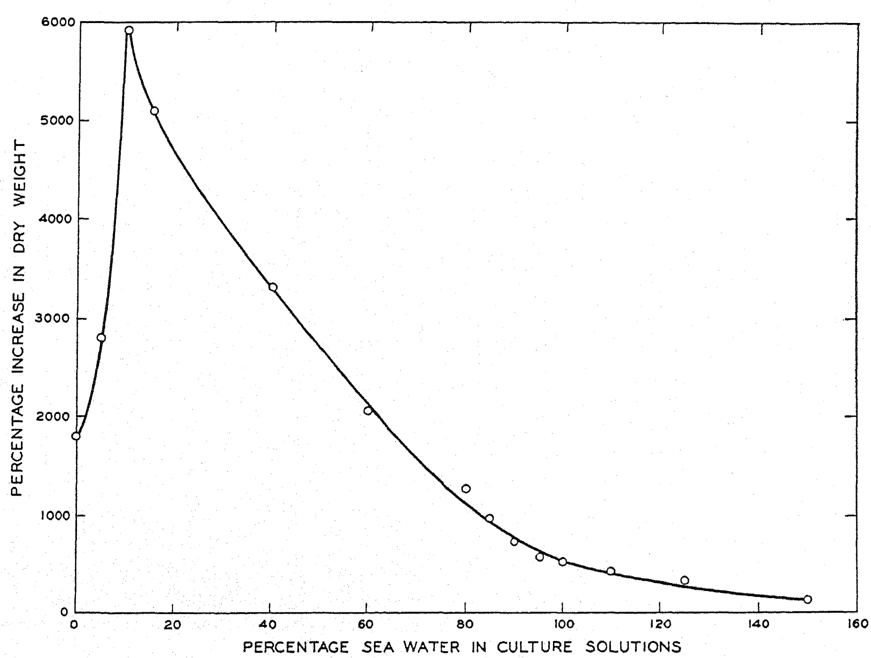

FIGURE 1. Growth of *Ruppia maritima* L. in different concentrations of sea water.

and carbon dioxide and oxygen supplies is shown in Figure 1. The results are presented in percentage increase in dry weight, using as a basis for calculation the percentage dry weights of comparable samples taken at the beginning of the experiments.

The optimum concentration of sea water for the growth of *Ruppia* was found to be 10 per cent, which is equivalent to a total salt concentration of 0.35 per cent. Growth of the plants in culture solutions containing from 5 to 60 per cent sea water, however, is shown to be greater than that found in the fresh water check culture. Except as shown by the dry weight measurements, no differences in the amount of growth could be observed in any of the cultures containing from 5 to 80 per cent sea water, as the plants in all cultures within those limits grew to the top of the water, produced seeds, and appeared to completely fill the culture vessels. In all cultures containing more than 80 per cent sea water, however, a decrease in the amount of growth was quite noticeable. The plants in any of the higher concentrations scarcely reached the top of the water, produced no seed, were spindling, and branched only from the roots. Yet, in all the cultures, including that containing 150 per cent sea water, the plants appeared entirely healthy and all made an appreciable amount of growth. This was quite noticeable from the thrifty appearing "runners" put out over the surface of the soil, even in the culture containing 150 per cent sea water.

DISCUSSION

The fact that *Ruppia maritima* L. will grow in water containing a relatively high salt content is not new. Osterhout (12) in 1906 grew the plant for 150 days in water from San Francisco Bay which contained a total salt content of 2.7 per cent, which is equivalent to 77 per cent standard sea water and is about the same as the salt content of Long Island Sound. Furthermore, the plants used by Osterhout were collected in water with a salt content fluctuating around 2.3 per cent, or approximately 65 per cent normal sea water. Some of his experimental plants also lived for 85 days in tap water and for 80 days in distilled water. Osterhout, however, did not determine the limits of sea water which *Ruppia* will tolerate. Graves (9) in 1908, working on the morphology of this plant, found that its roots could withstand 105 per cent sea water without becoming plasmolyzed. In 110 per cent plasmolysis was slow but finally distinct. He also observed that a 2.5 per cent solution of sodium chloride did not plasmolyze the leaves of *Ruppia*, but in a 3.0 per cent solution of this salt plasmolysis occurred in from 4 to 5 minutes. The ability of a plant such as *Ruppia* to withstand plasmolysis by sea water, however, undoubtedly will depend to a great extent upon the particular salt content of the medium in which the plant was originally collected. In this connection it is interesting to

note further some of the media in which *Ruppia* is known to grow. St. John and Courtney (13) observed *Ruppia maritima* L. growing in Epsom Lake, Washington, where the water is completely saturated with magnesium sulphate. They state (p. 105): "The liquid of Epsom Lake contains practically nothing but $MgSO_4 \cdot 7H_2O$ in a concentrated solution, so concentrated, in fact, that even the difference between the temperature of day and night will cause the salt to be crystallized out and exert enough force to split, from end to end, a metal pipe containing the liquid." Young (14, p. 387), writing on the physiography and condition of North Dakota lakes, states that the lakes "range all the way from those of a distinctly freshwater type, with low alkalinities, to exceedingly brackish waters with total carbonate alkalinities running up to 2000 ppm. and more." He observed that *Ruppia maritima* L. occurred abundantly in practically all the lakes of that region. Later he (15) found that *Ruppia* formed a definite zone in the main part of Devils Lake which contained a chlorine content of 1310 parts per million. Metcalf (11) observed *Ruppia* growing abundantly in the alkaline lakes of North Dakota. One of these, Lake Sheridan-Burleigh, in which *Ruppia* occurred in large quantities, contained a total salt content of 7.7 per cent, a concentration more than twice that of normal sea water. In these alkaline lakes, however, the percentage of sodium chloride in proportion to such salts as the sulphates of sodium, magnesium, and calcium, and the carbonate and bicarbonate of magnesium, and calcium, and the carbonate and bicarbonate of magnesium is found to be much lower than that in sea water. Evidently sodium chloride, which makes up about 80 per cent of the total salt content of the ocean, has a definitely greater inhibiting effect upon the growth of *Ruppia* than most of the other salts occurring in quantities in our natural waters. This inhibiting effect must be attributed to the specific toxicity of sodium chloride rather than to any osmotic factor, as Osterhout (12) has shown that a solution of this salt isotonic with sea water is fatal to *Ruppia*, but that the plant will live in sea water where sufficient amounts of other salts are present to counteract the toxic effects of the sodium chloride.

While it must be considered that the present experiments were carried out under favorable conditions in the greenhouse and that results so obtained are not always entirely applicable to natural situations, the evidence seems quite conclusive that *Ruppia maritima* L. will thrive in water containing a salt content not exceeding 80 per cent of the concentration of normal sea water. It is not believed that many of the bays, sounds, and estuaries along the Atlantic Coast have a total salt content greater than this amount, even the water directly touching the open coast does not contain a salt content equivalent to 100 per cent of what is considered the normal amount for the ocean. Undoubtedly, *Ruppia* will thrive in most of the bodies of water formerly occupied by *Zostera marina* L., but

it is extremely unlikely that *Ruppia* will tolerate as great a concentration of sea water as that formerly endured by *Zostera*, which until recent years was so abundant and so widely distributed along the Atlantic Coast. Probably, *Ruppia* could have thrived in the past on tidal mud flats but could not overcome the vigorous competition of the dense growths of the *Zostera*. The economic importance of *Ruppia* as a food plant for wild waterfowl and its ability to thrive in relatively high concentrations of sea water should merit its propagation in every available and favorable body of water along the Atlantic Coast.

SUMMARY

Experiments were carried on in the greenhouse to determine the sea-water tolerance of *Ruppia maritima* L., an important food plant for wild waterfowl. For the experiments, stock plants which had been growing for several months in culture solutions containing 20 per cent sea water were used. The plants thrived in concentrations of sea water from 0 to 80 per cent of the normal amount found in the ocean. The highest concentration in which the plants were found to thrive contained a total salt content equivalent to 2.8 per cent. While the plants lived and appeared healthy at the end of three months in concentrations up to 150 per cent sea water, or a total salt content of 5.25 per cent, they did not thrive or produce seeds. These results led to the conclusion that *Ruppia maritima* L. might be used advantageously in most coastal waters to replace *Zostera marina* L., which in recent years has almost completely disappeared from the Atlantic Coast.

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STORAGE TEMPERATURES AND CHEMICAL TREATMENTS FOR SHORTENING THE REST PERIOD OF SMALL CORMS AND CORMELS OF GLADIOLUS[1]

F. E. Denny and Lawrence P. Miller

Experiments on the effects of storage temperatures in shortening the rest period of plants have usually resulted in emphasizing the favorable effect of low temperatures. For example, the investigations of Coville (1) with blueberry, Weiss (9) with narcissus, Steinbauer (8) with Jerusalem artichoke, Luyten and Blaauw (6) with iris, Moore (7) with tulip, and Crocker (2) with seeds of various species agree in showing the favorable effect of low temperature storage, with the optimum usually as low as 10° C. or lower.

With large corms of gladiolus, however, Loomis and Evans (5) and Loomis (4) recommend high temperature storage (about 30° to 40° C.).

In a previous report (3) it was shown that success with the high temperature treatment of gladiolus was not attained in the period shortly after harvest when the corms were very dormant, but only in the later stages of the rest period. For investigating this interval between the beginning and the end of the rest period, small corms are more favorable than large ones because the dormant period is longer, and the importance of gradations in dormancy can be recognized more easily.

The present tests have been made, therefore, with small corms, and the tests were started shortly after harvest. The results show no exceptional behavior on the part of gladiolus, at least with the corm sizes used in these experiments, and emphasize the favorable effect not of high but of low temperatures in shortening the rest period. Temperatures of 3° and 10° C. during storage periods even as short as 28 days markedly hastened the germination when the stored corms were planted at room temperature. Only with corms that were not very dormant was 35° C. preferable to room temperature, and in such cases we must consider whether the high temperatures break the dormancy or merely hasten the growth of corms that, in reality, are no longer dormant.

Some treatments were made with vapors of ethylene chlorhydrin either before the corms were stored or after they had been stored at various temperatures. Small or negligible gains were obtained by treating corms that had been stored at low temperatures, but the chemical treatments were very effective in hastening the germination of corms which germinated poorly as a result of storage at room temperature or at higher temperatures.

Some tests with cormels also show a favorable effect of low temperature

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storage in shortening the rest period, good germinations resulting from cormels stored at 5° to 10° C. for 60 to 90 days and then planted at room temperature.

METHODS

After harvest the corms were stored in paper bags at room temperature until the storage period began. During the storage period at different temperatures the corms were mixed with moist sphagnum moss which was moistened at intervals thereafter. With the larger sizes 24 to 33 corms were used in each lot, and with the smaller sizes 50 to 100 corms. The temperatures at 3°, 10°, 29°, and 35° C. were obtained in large storage rooms equipped with regulating devices which maintained these temperatures closely. The room temperature was not regulated thermostatically but fluctuated from about 18° to 25° C.

For the treatments with vapors of ethylene chlorhydrin the corms were placed in quart Mason fruit jars into the top of which was spread loosely a piece of cheesecloth containing 2 cc. of 40 per cent ethylene chlorhydrin. The jar was closed and allowed to stand at room temperature for four days.

At the end of the storage and chemical treatment periods the corms were planted in soil in flats which were placed at room temperature. Germination counts were made at intervals of four to ten days, the sprouted plantlets being removed and discarded.

RESULTS

EXPERIMENTS WITH SMALL CORMS

The results with the No. 4 size corms (weight 6.3 g. each) are shown in Table I. It will be noted that the time after harvest at which the experiment was started was an important factor. Thus, with the Souvenir variety when the experiment was started 8 days after harvest, storage for 28 days at either 3° or 10° C. gave good germination in about 60 days after planting, but the corms stored at room temperature or 35° did not germinate well until about 100 to 120 days, and even at 120 days only about 11 per cent germination was obtained in the 29° lot. When the storage treatment was not started until 48 days after harvest, much earlier germinations were obtained from all of the lots, but here again the 3° and 10° lots showed gains of 40 days or more over the higher temperatures. The treatments starting 78 days after harvest were carried out at only three temperatures because of lack of sufficient number of bulbs for the 10° and 29° tests. At this stage of the rest period the favorable effect of high temperature storage becomes evident, 35° showing earlier germinations than either 3° or room temperature. However, the corms were not very dormant at

TABLE I

EFFECT OF STORAGE TEMPERATURE ON GERMINATION OF GLADIOLUS CORMS (SIZE NO. 4, WEIGHT 6.3 G. EACH)

Variety	Days after harvest when exp. started	Temp. (°C.) of storage for 28 days	% germ., days after planting						No. days for 50% germ.
			20 da.	40 da.	60 da.	80 da.	100 da.	120 da.	
Souvenir	8	3°		0	73	100			52
		10°		0	58	88	93	93	68
		Room				0	36	81	105
		29°					0	11	*
		35°				0	24	54	117
	48	3°	0	71	100				37
		10°	0	85	100				33
		Room	0	25	55	84	97		56
		29°	0	12	13	18	24		*
		35°	0	44	48	48	48		*
	78	3°	0	73	93	93			35
		Room	0	52	69	78			38
		35°	69	75	75	75			18
Alice Tiplady	8	3°		0	74	100			54
		Room			0	49	95	96	80
		35°			0	45	82	94	81
	48	3°	0	80	100				38
		Room	0	51	91	100			40
		35°	77	100					17
Remembrance	8	3°	0	89	100				35
		Room						0	*
		35°				0	7	7	*
	48	3°	0	100					23
		Room	0	6	10	12	12		*
		35°	0	17	21	21	21		*

* 50% germ. not reached at the end of the interval for which the last entry in the table is made.

this time as shown by the room temperature lot which gave 52 per cent germination 40 days after planting.

With the Alice Tiplady variety a somewhat similar behavior was obtained. In the test at eight days after harvest 3° was more favorable than higher temperatures, but in the tests started 48 days after harvest, when the corms were no longer very dormant, as shown by the germination of the room temperature lot, earlier emergence was obtained by high temperature storage.

The results with Remembrance variety show the favorable effect of 28 days' storage at 3° C., 100 per cent germination being reached in 40 to 60 days after planting, this result being obtained with corms so dormant that when planted 8 days after harvest no germination of the control lot

was obtained 120 days after planting, and when planted 48 days after harvest only 12 per cent emergence was reached in 100 days after planting. The 35° lots were slightly better than the room temperature lots in the tests with Remembrance, but they lagged far behind the 3° lots, not reaching 25 per cent germination in either test. Because of insufficient number of experimental bulbs a test at 78 days after harvest could not be made.

An experiment was carried out with some small corms (wt. 1.5 g. each) grown in the greenhouse from cormels. Ten days after harvest these were placed in storage at three different temperatures. At the end of a 28-day storage period some corms of each lot were planted at once in soil in flats which was stored at room temperatures, while other bulbs of each lot were treated with vapors of ethylene chlorhydrin and then planted. The results are shown in Table II. With all three varieties storage at 10° C. gave much earlier germination than storage at either room temperature or at 35°. The chemical treatment hastened the germination of all of the room temperature and 35° lots. It also gave a favorable response with the

TABLE II

EFFECT OF STORAGE TEMPERATURE AND CHEMICAL TREATMENT ON GERMINATION OF GREENHOUSE-GROWN GLADIOLUS CORMS
(SIZE NO. 5–6, WEIGHT 1.5 G. EACH)

Variety	Chemical treatment	Temp. (°C.) of storage for 28 days	% germ., days after planting						No. days for 50% germ.
			40 da.	60 da.	80 da.	100 da.	120 da.	140 da.	
Souvenir	Not treated	10°		0	55	71	71	71	78
		Room				0	20	71	128
		35°				0	9	52	137
	Ethylene chlorhydrin	10°	0	53	91	98	98	98	58
		Room			0	14	66	86	111
		35°	31	42	50	55	61	76	80
Alice Tiplady	Not treated	10°	81	89	89	89	89	89	35
		Room				0	32	40	*
		35°		0	11	44	68	81	106
	Ethylene chlorhydrin	10°	86	93	93	93	93	93	35
		Room	54	85	89	89	89	89	39
		35°	81	93	93	93	93	93	32
Remembrance	Not treated	10°			0	35	63	85	128
		Room				0	10	46	*
		35°				0	10	51	139
	Ethylene chlorhydrin	10°	25	75	93	93	93	93	50
		Room		0	14	31	79	81	109
		35°			0	15	67	74	114

* 50% germ. not reached at end of the interval for which the last entry in the table is made.

Souvenir and Remembrance corms whose dormancy had been partially broken by the storage at 10° C. With the Alice Tiplady lots, however, storage at 10° for 28 days resulted in 81 per cent germination in 40 days after planting, and treatment with the chemical at the end of the storage period produced no further gain.

Evidence for the favorable effect of low temperature storage and of chemical treatment is found in Table III. This experiment was started 8

TABLE III

EFFECT OF STORAGE TEMPERATURE AND CHEMICAL TREATMENT ON GERMINATION OF GLADIOLUS CORMS, VAR. SOUVENIR

(SIZE NO. 6, WEIGHT 1.12 G. EACH)

Chemical treatment	Temp. (°C.) of storage for 35 days	% germ., days after planting					No. days for 50% germ.
		40 da.	60 da.	80 da.	100 da.	120 da.	
Not treated	3°	0	51	64	71	72	58
	10°	0	51	67	73	73	58
	Room		0	12	21	22	*
	29°		0	1	6	9	*
	35°		0	1	4	11	*
Ethylene chlorhydrin	3°	55	80	85	85	85	38
	10°	56	59	64	64	64	36
	Room	38	75	87	88	90	45
	29°	9	44	77	88	90	63
	35°	11	50	80	83	85	60

* 50% germ. not reached at the end of the interval for which the last entry in the table is made.

days after harvest, with No. 6 corms of the Souvenir variety. The bulbs were very small, average weight 1.12 g. each. The storage period was 35 days at the end of which some of the corms from each temperature lot were planted at once while others were treated with vapors of ethylene chlorhydrin and then planted. These bulbs were very dormant, the germination of the control lot stored at room temperature reaching only 22 per cent after 120 days from the time of planting. The germination of the lots stored at 29° C. and 35° C. before planting was even slower, values of only 9 and 11 per cent being reached in 120 days. In contrast to these results with high temperature storage were those obtained by storage at 3° and 10° C. which resulted in 50 per cent germination in about 60 days. The chemical treatment hastened the germination of the bulbs irrespective of the temperature at which they had been stored previous to the treatment. Even bulbs subjected previously to the favorable influence of storage at 3° and 10° C. responded to chemical treatment, and a still further gain of about 20 days resulted. It is with the corms stored at room temperature, 29° and 35° C., however, that the chemical treatments produced the largest gains. Germination percentages reached by the untreated lots in

120 days were reached by the treated lots in 40 days. Thus, the unfavorable effects of high temperature storage were overcome by treatment of the stored corms with vapors of ethylene chlorhydrin.

In the experiments that have just been described the corms were stored at certain temperatures for definite periods (28 and 35 days). In the experiment now to be described the bulbs were stored continuously for longer periods at the different temperatures, and samples were removed for planting at intervals after the start of the storage period. The corms were the No. 6 size (average weight 1.0 g. each) and the experiment was started 7 days after harvest. The results are shown in Table IV. With the Alice

TABLE IV

EFFECT OF DURATION OF THE TEMPERATURE OF STORAGE ON GERMINATION OF GLADIOLUS CORMS

(SIZE NO. 6, WEIGHT 1.0 G. EACH)

Variety	No. days stored before planting	Temp. (°C.) of storage	% germ., days after planting						No. days for 50% germ.
			20 da.	40 da.	60 da.	80 da.	100 da.	120 da.	
Alice Tiplady	36	3°		0	8	52	85	93	79
		10°			0	46	88	97	81
		Room			0	3	6	15	*
		29°						0	*
		35°				0	3	6	*
	66	3°	0	23	87	97	97		45
		10°	0	36	97	100	100		42
		Room			0	8	18		*
		29°	6	6	6	6	6		*
		35°	6	6	6	6	6		*
	97	3°	0	97	97				24
		10°	31	93					24
		Room	15	26	33				*
		29°	46	48	48				*
		35°	85	97	97				16
Remembrance	36	3°	0	84	94	100	100	100	32
		Room			0	3	3	3	*
		35°					0	11	*
	66	3°	92	100	100	100	100		19
		Room					0		*
		35°					0		*
	97	3°	96	100					10
		Room			0				*
		35°		0	6				*

* 50% germ. not reached at the end of the interval for which the last entry in the table is made.

Tiplady variety, samples removed after 36 days at either 3° or 10° C. showed 50 per cent germination in 80 days after planting; the lots stored at room temperature, 29° C. and 35° C., however showed germination

percentages of only 0 to 15 at the expiration of 120 days from planting. When the storage period was lengthened to 66 days the low temperature lots made a still further gain in earliness of germination, reaching the 50 per cent stage in about 45 days; the lots stored at the higher temperatures failed to germinate satisfactorily, showing germination percentages of only 6 to 18 after 100 days. After a storage period of 97 days, it will be observed that the bulbs had lost much of their dormancy at any storage temperature, and under these conditions the 35° lots germinated in advance of those stored at other temperatures, the gain over 3° being not great but that over room temperature being appreciable.

With the Remembrance variety the superiority of low over high temperature storage for breaking dormancy is very striking, even 36 days at 3° C. being sufficient to induce rapid sprouting when the corms were

TABLE V

EFFECT OF LOW TEMPERATURE STORAGE ON THE GERMINATION OF GLADIOLUS CORMELS

No. days stored before planting	Temp. (°C.) of storage	Var. Souvenir		Var. Alice Tiplady		Var. Remembrance	
		No. days for 50% germ.*	% germ. after 6 months	No. days for 50% germ.*	% germ. after 6 months	No. days for 50% germ.*	% germ. after 6 months
30	5°	90	79	**	30	**	29
	10°	87	83	**	32	170	40
	15°	100	67	**	35	**	17
	Room	122	77	240	21	**	9
61	5°	65	82	162	45	33	55
	10°	60	81	**	34	26	81
	15°	65	67	149	43	**	32
	Room	95	83	226	26	**	17
92	5°	45	90	76	71	30	71
	10°	46	91	140	84	26	75
	15°	46	76	106	50	167	40
	Room	78	68	189	29	**	20
120	5°	41	98	22	76	23	76
	10°	38	98	105	60	21	90
	15°	53	75	99	78	60	54
	Room	94	79	163	51	**	21
151	5°	38	92	53	82	25	80
	10°	30	91	66	79	19	80
	15°	42	73	54	84	51	64
	Room	80	75	136	52	**	12
181	5°	—	—	50	98	—	—
	10°	—	—	51	89	—	—
	15°	—	—	59	95	—	—
	Room	—	—	104	79	—	—

* No. days from date of planting.
** 50% germ. not reached.

planted at room temperature; but the lots held at either room temperature or 35° for periods of 36, 66, or 97 days either failed to germinate at all, or showed only low percentage germinations.

EXPERIMENTS WITH CORMELS

The favorable effect of low temperature storage upon the germination of gladiolus cormels is shown in Table V. The experiments were started November 16, 1933 with cormels of the 1933 crop. The cormels were passed through screens and the ones used in these tests were those which passed through a screen with square openings measuring 5/16 inch on a side but which failed to pass through a screen with openings 4/16 inch on a side. Each lot consisted of 100 cormels.

During the period of storage the cormels were placed both in cheesecloth bags and in soil in flats. Only the results from the lots in cheesecloth bags are given in Table V. The results from those stored in soil were similar in all respects.

The most favorable temperatures were 5° and 10° C., these being in all cases much better than room temperature, and in most cases better than 15° C.; with Alice Tiplady 5° was definitely better than 10° C. The time required at these favorable temperatures in order to induce good sprouting varied with the variety. The Alice Tiplady cormels were very dormant and required 92 days or more of storage at 5° C.; Remembrance cormels responded to storage for 61 days at 5° or 10°; and Souvenir which was less dormant gave a gain of about 30 days over the corresponding controls after 30 to 61 days at 5° or 10° C.

SUMMARY

1. Small gladiolus corms (sizes No. 4 to 6, wt. 1 to 6 g. per corm) of the varieties Souvenir, Alice Tiplady, and Remembrance were stored at temperatures varying from 3° to 35° C. for periods varying from 28 to 97 days and records were made of rate of germination when the stored corms were planted in soil at room temperature.

2. Low temperatures, 3° and 10° C., were effective in shortening the rest period, and were distinctly more favorable than higher temperatures such as room temperature, 29° C., or 35° C.

3. Storage at 35° C. gave germinations earlier than at room temperature only in the later stages of the rest period.

4. Ethylene chlorhydrin treatments of corms that had been stored at different temperatures hastened germination, giving large gains if the corms had been stored previously at room temperature or higher, but usually only small gains if they had been stored at low temperature.

5. Low temperature storage of cormels was effective in shortening the

rest period, good germinations resulting from storage of cormels at 5° to 10° C. for 60 to 90 days.

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PROPAGATION OF LYCOPODIUM. I. SPORES, CUTTINGS, AND BULBILS

FLORENCE L. BARROWS

INTRODUCTION

Interest in propagation of *Lycopodium* seems to date back to 1794, when Lindsay (44, p. 100), an English surgeon, who had noted very young ferns growing in the open in Jamaica, sowed not only fern spores, but also those of moss and *Lycopodium*. "I have very lately sown that fine farina or dust contained in the anthera of a species of the genus *Bryum*, *viz. Bryum cespiticium*, or one very like it, and also the farina of *Lycopodium cernuum*. There is a vegetable growth taking place where they were sown, which I hope will prove their young plants." So stated John Lindsay, surgeon in Jamaica, in a communication to the Linnean Society, read by Sir Joseph Banks, Jan. 3, 1792. Unfortunately he left no figures of *Lycopodium*, although he has several of fern prothallia showing development up to the second leaf of the sporophyte. On Apr. 2, 1793, Sir Joseph (45, p. 313) read to the Society an extract from a letter dated June 30, 1792, in which the Jamaican surgeon says, "In my last letter I mentioned I had sown some of the fine dust or farina from the fructification of *Lycopodium cernuum*, and that from the capitula, generally called antherae, of *Bryum caespititium*, or a species very like it, but I then thought their success somewhat doubtful. I have since repeatedly sown them both, and in a proper situation find they grow very readily."

The competition for priority was evidently as keen then as now and this elicited some "Additional remarks" by James Edward Smith, M.D., P.L.S. (53, p. 314). "The raising of any species of *Lycopodium* from its farina has not to my knowledge been described as practicable; Mr. Lindsay, therefore, has all the merit of an original observer. It is not to detract from his due praise, but to do justice to unostentatious ingenuity, that I now mention Joseph Fox, a journeyman weaver of Norwich, as having made similar experiments with *Lycopodium Selago* with the like success. He shewed me, in the year 1779, young plants of this species raised from seed in his own garden."

Other investigators have not always been so successful in their attempts to make *Lycopodium* spores grow. Table I sums up, very briefly, some of these efforts. It is based on the historical summaries of Chamberlain (15) and Rayner (50), as well as on the original literature.

As the discovery of prothallia has stimulated attempts at spore germination, especially by Treub (62, 65, 66) and Bruchmann (11, 13), the records of prothallia are put into a separate table for comparison (Table II). Hegel-

TABLE I

LYCOPODIUM SPORE GERMINATION

Date	Author	Species	Germination
1779	Fox (53)	*Selago*	Norwich, England reported to Linnean Society.
1794	Lindsay (44, 45)	*cernuum*	Grew readily in Jamaica.
1842	Spring (60)	*clavatum* *complanatum*	Failure attributed to "existence of male plants only." Bruxelles.
1848	Spring (60)	*clavatum*	Unsuccessful attempt by Dr. Oschatz of Berlin.
1851	Hofmeister (32)	*clavatum* *inundatum* *Selago*	Predicted prothalli but secured no spore germination after repeated trials.
1858	De Bary (3)	*inundatum*	Sept. 1855, grew in 9 days. Mar. to May, 1856, on soil, to 7-cells. Only one to 11-cells.
1873	Fankhauser (22)	*annotinum*	2-cell stage only. Switzerland.
1880	Beck (4)	*inundatum*	Oct. to mid-Nov., 1-1/2 mos. Only 1 spore grew; giving 10-cells. Rest increased in volume.
		clavatum	Remained green and looked viable for 2 yrs., but did not germinate.
		alpinum *annotinum* *Selago*	Also negative results on various media, with different light and temperature conditions.
1884	Treub (62)	*cernuum*	Few days on soil; 4 to 6 weeks on tree trunks, Java. Antheridial stage must have fungus, "probably *Pythium*" to develop.
1886	Treub (63)	*Phlegmaria*	Unable to germinate spores, but some sown on tree trunks, 1882, gave "plantules," Dec. 1883.
1887	Treub (64)	*densum* type	Grew in few days. Form primary tubercle.
		curvatum	Lived 8 to 10 mos., then ceased development.
1888	Treub (65)	*salakense*	Sown Jan. 17 in lab., grew in few days. Did not develop beyond tubercle stage without fungus.
1910	Bruchmann (13)	*Selago*	3 to 5 yrs. to germinate, Gotha. 6 to 8 yrs. to form gametes. 4 to 5-cells without fungus.
		annotinum *clavatum*	6 to 7 yrs. to germinate. 12 to 15 yrs. to form gametes.

maier's (30) anatomical studies of *Lycopodium*, and Wigglesworth's (68) examination of some of Bruchmann's material, do not indicate that either noted the presence of fungus in sporophytes.

Repeated references are found in the literature in regard to the difficulty of germinating *Lycopodium* spores. The time reported for spore germination seems to range from nine days for *L. inundatum* [De Bary (3) in 1858] to six to seven years for *L. annotinum* and *L. clavatum* [Bruchmann (13) in 1910] with the development of antheridia and archegonia after 12 to 15 years.

In a recent taxonomic discussion of the spores of the genus *Lycopodium* in the United States and Canada, L. R. Wilson (71, p. 17) notes the similarity of spore pattern of *L. complanatum*, *L. flabelliforme*, and *L. tristachyum* in Wisconsin, where the three forms merge, and "appear to be ecologically controlled. Victorin has suggested hybridization for the origin of many forms in this group, but thinks this has never been demonstrated, and the difficulties which accompany the germination of *Lycopodium* spores make such experiments practically impossible." [See Marie-Victorin (47).]

GERMINATION OF SPORES

The present experiments were started in September 1933. A limited supply of fresh spores of *Lycopodium obscurum* L. was available. These were sown on Sept. 10, in distilled water, Beyerinck solution modified (25, p. 102), 2 per cent sucrose, 2 per cent maltose, and duplicate sets were placed in light and in darkness.

A limited number of *L. clavatum* L. spores was available, and these were also run in duplicate sets in light and in darkness, in distilled water, Beyerinck modified, Beyerinck modified plus 2 per cent agar, peat and sand (31), peat, Connecticut soil (from a *Lycopodium* station), and live sphagnum moss, and at the following temperatures: room, 5°, 10°, 15°, and 20° C.

A larger quantity of fresh spores of *L. complanatum* var. *flabelliforme* Fernald was available. [This variety is the *L. flabelliforme* of Blanchard and Small, and will be abbreviated in the rest of this paper as *L. complanatum* (21, 24).]

MEDIA AND TEMPERATURES

The following media and temperatures were used: Beyerinck solution, and agar at room temperature, 5°, 10°, 15°, and 20° C.; peat, peat and sand at room, 10° and 20° C; and sphagnum, Beyerinck solution, distilled water, and 2 per cent sucrose at room temperature.

The main harvest of *L. complanatum* spores was made the last of September when the cones were shedding freely. On Sept. 30, a large number of spores were stored in gelatin capsules placed on absorbent cot-

TABLE II

RECORDS OF LYCOPODIUM PROTHALLIA AND SPORELINGS*

Date	Author and place	Species	Germination
1875	Fankhauser (22) Switzerland	*annotinum*	Sept. 1872, prothallia subterranean and lack chlorophyll. 13 sporelings 3 to 18 cm. high.
1884	Treub (62) Java	*cernuum*	Early antheridial stage. *Pythium* in prothallus.
1885	Bruchmann (10) Gotha	*annotinum*	3 prothallia with antheridia, Aug. 4. "Pilze vom Klasse der Chytridien."
1886	Treub (63) Java	*Phlegmaria*	Endophyte in prothallia.
1887	Treub (64) Java	*cernuum*	Over 100 prothallia collected.
1887	Goebel (27)	*inundatum*	Endophytic fungus, probably *Pythium*, in both prothallia and sporophyte.
1888	Treub (65) Java	*carinatum* *nummularifolium* *Hippuris*	
1889	Kuhn (41)	*inundatum*	Fungus mycelium intracellular.
1898	Bruchmann (11) Gotha	*annotinum* *clavatum*	Lacked early stages. Had gametes and embryos. "Sphaeromen" in *clavatum*.
1908	Bruchmann (12) Gotha	*complanatum*	Wigglesworth (68) studied young sporophytes in 1907. No fungus reported.
1910	Bruchmann (13) Gotha	*Selago*	"Sporangioles" in *Selago*. Endophyte different in *clavatum* and *Selago*.
1899	Lang (42) Scotland	*clavatum*	July 1898. 7 prothallia, 3 with young plants. Endophytic fungus non-septate with "multinucleate vesicles," possibly *Peronospora*; no fungus in sporelings.
1916–1917	Holloway (33, 34) New Zealand	*ramulosum*	April, 24 prothallia and young plants, probably developed in 1 season. No fungus.
		Billardieri	Prothallia found in January. No fungus.
		laterale	Summers, 1905 and 1914; young plants and 8 prothallia. No fungus.
		cernuum	Young plants and prothallia; "seem to germinate very freely." No fungus.
		densum	2 young plants, one with large foot.
		fastigiatum	61 prothallia found during 3 yrs. "possibly 15 yrs. for development."
		scariosum	About 60 prothallia. Propagated freely from spores. Fungus in cortical tissue.
1917	Spessard (56, 57) Marquette, Michigan	2 *annotinum* 8 *clavatum* 7 *complanatum* 1 *lucidulum* 3 *obscurum*	21 prothallia and over 50 sporelings of 5 species; first record for America. Fungus noted.

* Petry has a list of several stations not yet published.

TABLE II (*Continued*)

Date	Author and place	Species	Germination
1917	Chamberlain (15) New Zealand	3 *laterale*	Prothallia and sporelings with protocorm, collected by A. P. W. Thomas, University of Auckland.
		3 *scariosum*	Dense fungus.
		9 *volubile*	And 2 sporelings. Endophytic fungus.
1919	Holloway (35) New Zealand	*Billardieri* var. *gracile*	"very large number of prothalli."
		varium	"about a dozen."
		volubile	Prothallus growing on surface becomes green. Cortical fungus layer 2 to 4 cells wide.
		fastigiatum	Fungus present.
		ramulosum	Young prothallia green and free from fungus for considerable period.
1920	Holloway (36) New Zealand	*Billardieri*	Fungus present.
		Billardieri var. *gracile*	Intercellular fungus "spores" in cells of prothallus; but no fungus in epidermis.
		cernuum	Fungus present.
		laterale	Fungus present.
		ramulosum	Fungus present. Above ground, prothallus is green.
1922	Spessard (58) Marquette, Michigan	*lucidulum*	Endophytic fungus different in 2 species.
		obscurum var. *dendroideum*	"Probably not *Pythium*"; "may both be Ascomycetes."
1924	Stokey and Starr (61) Massachusetts	*clavatum* *complanatum* *obscurum*	Endophytic fungus. 11 stations.
1924	Degener (16) Massachusetts	*clavatum*	Sporelings, Mar. 29, 1922.
		complanatum	
		obscurum var. *dendroideum*	May to June, 1922, several hundred under hemlocks. Mycorrhiza on hemlocks, and a fungus, probably *Tremellodendron*.
1924 1925	Degener (17) Hawaii	*cernuum*	Hundreds of prothallia and sporelings, most numerous at 31° C. in active crater of Kilauea.
1928	Spessard (59) Michigan	*lucidulum*	Several hundred sporelings from 1 mm. to 4 cm. found 1918. "Prothallus seems to require fungus and will not grow without it."
1933	Barrows Union, Connecticut	*complanatum*	Aug. 29, 1 sporeling with prothallus attached.
		clavatum	Sept. 23, 2 sporelings, one with prothallus

ton in wide-mouth bottles. The top was covered with paraffin paper, held in place by an elastic band, to exclude dust and prevent excessive loss of moisture. Capsules were stored at room temperature in light and in darkness, and also in the dark in constant temperature ovens at 1°, 5°, 10°, 15°, 20°, 30°, and 35° C., and at one alternating temperature of 5° to 25° C. Sample capsules were removed in about two weeks and thereafter at about monthly intervals, and sown on media as follows: Oct. 16 on Beyerinck agar; Nov. 21 on Beyerinck agar, and 2 per cent maltose; Dec. 21, 1933, and Jan. 22, 1934, on Beyerinck agar, and peat; Feb. 22, Mar. 22, Apr. 21, May 21, and Aug. 2 on peat.

On Jan. 8, 1934, spores which had been held at low temperature around 0° C. in the ante-chamber to the freezing room, since Nov. 6, 1933, were started on the following alternations: freezing room to 10° C. Jan. 8 to Mar. 8; 0° to 10° C. Jan. 8 to Mar. 8.

Samples were removed and sown on peat on March 8, 1934, and the alternating temperatures were continued until June 15.

Results. One set of spores of *L. complanatum* showed germination. This was the lot stored at the different temperatures ranging from 1° to 35° C. and alternating from 5° to 25° C. for a period of approximately five months from Sept. 30, 1933, to Feb. 22, 1934. These were sown on 4-inch petri plates of moist peat and kept in a copper case in a dark locker at room temperature. Just about a year after sowing, i.e., Feb. 22, 1934, to Feb. 23 to 25, 1935, germination was discovered in all the cultures of this one set. Table III gives the results.

TABLE III

L. COMPLANATUM SPORES STORED FOR 5 MONTHS AT DIFFERENT TEMPERATURES; GERMINATION ON MOIST PEAT. FEB. 1934 TO APR. 1935

Storage temperatures	No. germ.*	No. not germ.*	Per cent germ. Feb.	Per cent germ. Apr.
1. Room, light	25	299	8.3	10.9
2. Room, dark	26	208	12.5	13.7
3. Oven, 1° C.	24	106	10.6	19.5
4. Oven, 5° C.	25	232	10.7	14.1
5. Oven, 10° C.	27	214	12.6	13.2
6. Oven, 15° C.	13	166	7.8	14.2
7. Oven, 20° C.	22	188	11.6	16.2
8. Oven, 25° C.	16	176	9.1	7.3
9. Oven, 30° C.	2	171	1.1	7.8
10. Oven, 35° C.	29	295	9.8	8.4
11. Alternating 5° to 25° C.	30	184	16.3	18.3

* Counts based on 10 fields of the microscope.

In most of these cultures fungi were present; some small motile bodies, dark brown resting spores, and some slender hyphae. The fungus relationship will be discussed later. Several stages of development were found in

the germinating spores (Figs. 1 and 2). There seemed to be little difference in the rate of development in the spores which had been subjected to the different temperature treatments. The percentage of germination varied from 1.1 to 16.3 per cent. These percentages are based on an average of counts of ten fields of the microscope, totals ranging from 130 to 334 spores. Actual figures may be obtained by adding columns 2 and 3 in each case. As Table III indicates, the best germination was in the spores subjected to alternating temperatures, 5° to 25° C., but it was not remarkably higher than those from room temperature, 10°, or 20° C. The low germination at 30° C. may not be significant, as at 35° C. germination was nearly as good as at several lower temperatures, such as 20° and 25° C. The discrepancy may be due to experimental error as there were fewer spores present in this culture than in the rest of the set, and the gelatin capsule in which they were stored was rather opaque and damp at the time they were sown.

It is difficult to see all the planes of cell division through the spore coat, which darkens with age, but early stages were found closely resembling the method of cell division shown by Bruchmann's figures for *L. clavatum* (13, p. 229, Fig. 1), *L. annotinum* (13, p. 229, Fig. 2), and *L. Selago* (13, p. 232, Fig. 3). The *L. complanatum* spores in these cultures seemed to have reached stages varying from two to three cells to at least eight or ten cells (see Fig. 1 A or B; Fig. 2 A).

These same cultures of *L. complanatum* spores were examined again on April 29, 1935, and in all cases but two showed a higher percentage of germination than in February. Some fungus hyphae were present; small motile cells were quite numerous in several cultures, and at least one spherical fungus body contained a number of actively motile small cells. In the young gametophytes small oil globules were abundant, especially along the newly-formed cell walls. In some cases cell division had advanced beyond that of the same cultures in February. The maximum number of cells seemed to be about ten. In no case was the fungus seen to have penetrated the gametophytes, although hyphae were present on the surface of several young prothallia.

May 16 to 17, 1935, the percentage of germination was about the same in these cultures with one exception. The spores stored under alternating temperatures showed 22 per cent germination. This lot showed the highest germination throughout the experiment.

HUMIDITY

Use was made of humidity chambers, which are large glass desiccators containing different concentrations of sulphuric acid, prepared according to the method of Robert E. Wilson (72).

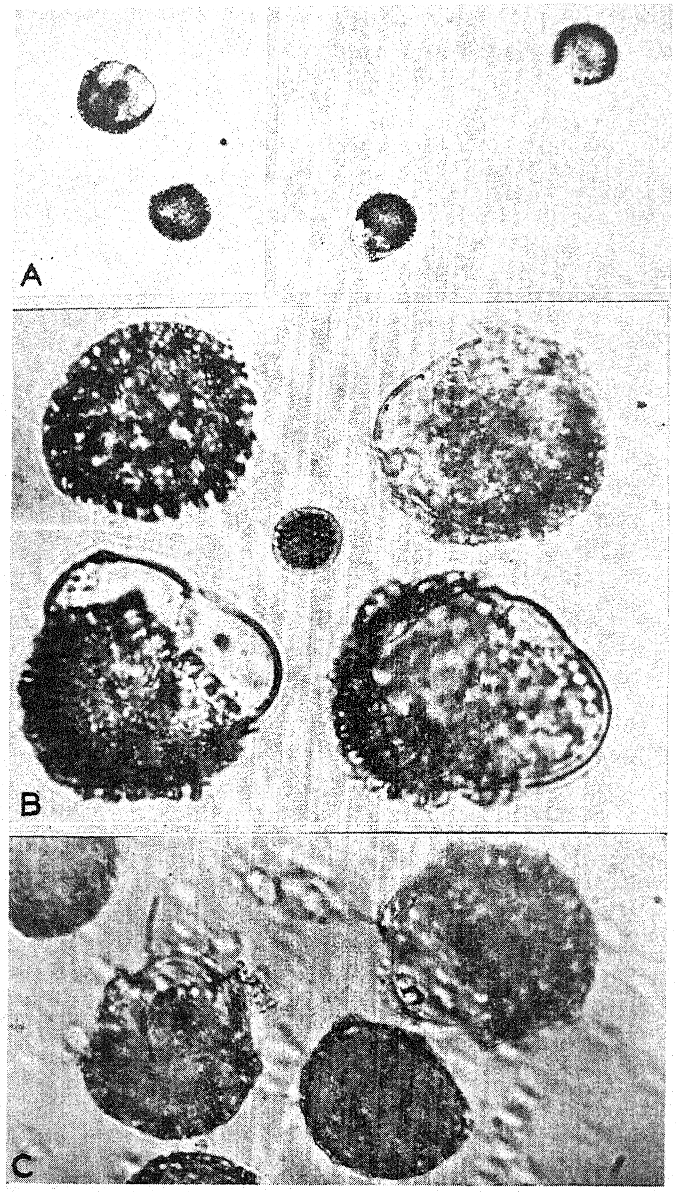

FIGURE 1. Germinating *Lycopodium* spores. (A) *L. complanatum.* × about 320. (B) *L. complanatum.* Center, fungus spore. × about 1120. (C) *L. obscurum.* × about 920.

Freshly-shed spores of *L. complanatum* were stored in gelatin capsules. Strobili or cones were also placed in glass vials covered with coarse muslin held over the mouth by an elastic band. *L. lucidulum* Michx. tips bearing sporangia were placed in vials and also a limited supply of *L. obscurum* spores in cones. The vials were placed in wide-mouth glass bottles and stored in the humidity chambers at room temperature in light, on Nov. 6, 1933. In the drier chambers, the *L. complanatum* spores were soon shed from the cones, which had been placed in an inverted position. The quantity of *L. obscurum* spores was so limited as to permit only a few tests. Three sets of cultures of this species were started after intervals of one week, two weeks, and one month.

L. complanatum spores from both capsules and cones, and *L. lucidulum* spores were removed at the following intervals: after 1 week, on Nov. 13, 1933, and 2 weeks on Nov. 20, to Beyerinck agar, and sphagnum; 1 month, Dec. 4, to Beyerinck agar plus 2 per cent maltose, and peat; 2 months, Jan. 2, 1934, to Beyerinck agar, and peat; 3 months, Feb. 1 to Beyerinck agar, and potato dextrose agar; 4 months, Mar. 1; 5 months, April 4; 6 months, May 1; 7 months, June 1; and 9 months, Aug. 1 to peat.

At time of removal from humidity chambers, spores were examined microscopically for structural changes, before sowing. The oil content will be discussed later. With a sterile platinum needle it is fairly easy to recover spores from media. Thousands of spores have been examined. Most look normal and healthy, and like Beck's, in 1880 (4), look viable, but do not germinate.

Results. On Jan. 14, 1935, some *L. obscurum* spores were found to have split their coats. These spores were collected at Woody Corners, near South Hadley, Massachusetts, Oct. 31, 1933. The cones were not yet shedding. They were placed in the various humidity chambers for two weeks, Nov. 6 to Nov. 20, 1933. In dry chambers, the spores were shedding by the end of this period. Spores were sown on Nov. 20, on damp sphagnum in 4-inch petri plates and were placed in a dark locker. On Mar. 6, 1934, distilled water was added to replace loss by evaporation, and the plates put into a copper case and returned to the locker until Jan. 1935. On Jan. 12, they were removed to the laboratory and Jan. 14 to 15, 1935, some split spore coats were discovered in all the cultures except from humidity No. 5, 35 per cent moisture, where too few spores were recovered to determine whether any had germinated (see Fig. 1 C and Fig. 2 B).

The spores which were open had burst at the tri-radiate crest, and the contents protruded through the opening as shown in Figures 1 and 2. The pattern of the spore coat has been omitted, but the thickness of the spore wall has sometimes been indicated. The spore contents were so filled with small oily-looking globules or plastids that it was difficult to see any cell divisions. Some fungus hyphae, some spherical fungus bodies, and nu-

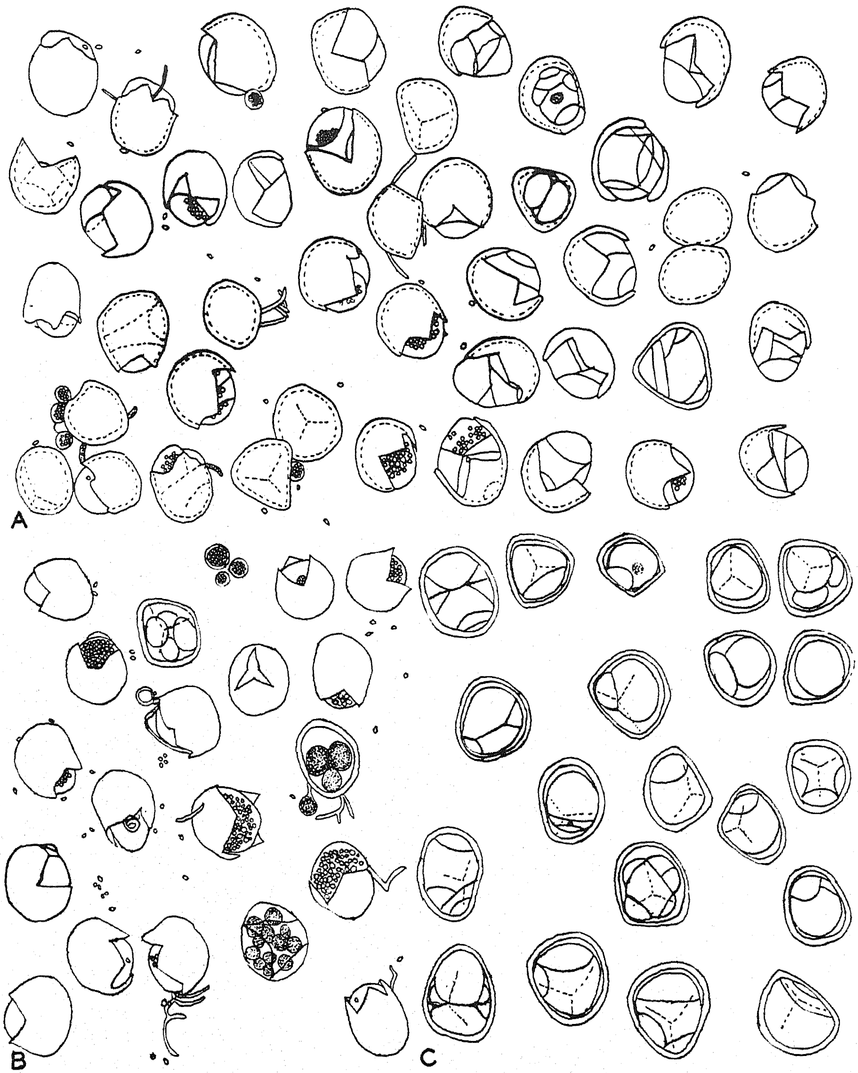

FIGURE 2. Germinating *Lycopodium* spores with aid of drawing ocular. × about 555. (A) *L. complanatum* after 5 months' storage at different temperatures; on peat 12 to 14 months. (B) *L. obscurum* on sphagnum 14 months. (C) *L. obscurum* washed in 95 per cent alcohol 2 minutes. Germination in 2 per cent maltose in 27 to 37 days.

merous small motile bodies were present in most of these cultures. The numbers of germinating spores were small and no counts were made. There seemed to be little difference in the spores subjected to differences in humidity during the two-week treatment in November.

One other species showed a few cell divisions. *L. lucidulum* spores stored over concentrated sulphuric acid, in humidity No. 9, dry, from Nov. 6, 1933 till Aug. 1, 1934, nearly nine months, were sown on peat. On Oct. 27, 1934, several spores showed a division into two cells.

On April 15, 1935, the *L. obscurum* spore cultures from the humidity tests were examined again and found to show more advanced stages of cell division than three months earlier in January. The best developed gametophytes contained many small oily globules, which in many cases were densely massed along the newly-formed cross walls. Small motile cells were quite numerous and occasional spherical fungus bodies were present. About six prothallia were found in which fungus hyphae had penetrated the gametophyte or were entering between the opened spore coat and the growing prothallium.

ALCOHOL TREATMENT

Another test on *L. obscurum* spores was made in the fall of 1934. These cones were collected in Union, Connecticut, Nov. 4, 1934, after some hard frosts, and were stored in the cool room at the Institute, Nov. 7 to 8. On Nov. 8, they were shaken vigorously for two minutes in vials of 95 per cent alcohol, which was filtered off through Whatman filter paper. Some oil was dissolved out as shown by the film left when the alcohol was allowed to evaporate in beakers. The spores were washed off the filter paper with distilled water into flasks of sterile distilled water, or other media. Controls were washed in the same way in distilled water, and otherwise treated as the first set. Various culture media were used as follows: sterile distilled water, 2 per cent maltose, potato dextrose agar, native soil, moist sphagnum, and moist peat. All were kept in the light in the laboratory at room temperature. *L. annotinum* and *L. complanatum* spores were also washed in 95 per cent alcohol, and controls in distilled water, and cultured. Fungus spores and hyphae are usually present on the surface of *Lycopodium* spores. The alcohol treatment did not remove all of these, and some cultures became rapidly overgrown with fungi. The *L. obscurum* spores and a few of the *L. annotinum* spores were the only ones which had yet shown cell division.

On Dec. 5, 1934, the first cell division was noted in *L. obscurum*. The alcohol-washed spores grown in distilled water, showed 1 and 2-cell stages within the spore coat, while the 2 per cent maltose culture showed 1, 2, and 3-cell stages. On Dec. 6, the native soil also showed 1, 2, and 3-cell stages, while on sphagnum there were only 1 and 2-cell stages. Dec. 7,

the peat gave 1 to 4-cell stages, and showed a few spore coats cracked open. By Dec. 15 to 17, the maltose culture showed further divisions from 4 to 8-cell stages, and on Jan. 16, 1935, one was found which had reached about a 10-cell stage. Live fungus was present, showing hyphae, with round fruiting bodies, conidia, and also small motile cells. Thus about a month after the alcohol treatment, the spores of *L. obscurum* showed cell division in distilled water, 2 per cent maltose, native soil, moist sphagum, and moist peat (see Fig. 2 C).

NATURE OF THE SPORE COAT

Beck (4) as early as 1880 noted that the spore coat of *Lycopodium* had three layers. Baranov (2) studied the development of the spore coat of *L. clavatum*. Zetzsche and Huggler (74), Zetzsche and Vicari (76), Zetzsche and Kälin (75), and Kirchheimer (39) have made extensive studies on the spores of *L. clavatum* in connection with work on pollens and spores in coal. Spores of this species were subjected to heat up to 340° C. for periods up to 15 hours and to chemical agents. Kirchheimer's figures (39, 40) show the resistance of the spore wall and the permanence of the typical netted pattern. He finds (39, p. 185) that "spores of *Lycopodium clavatum* sulphurated during heat, are only slightly altered in size, while its structure sustains injury. Sulphuration effects rigor of the formerly elastical exospore and thus forwards mechanical injuries. The spores are indifferent to the application of weak macerating agents, a strong oxident, however, destroys them completely."

Zetzsche and Huggler (74) extracted from *L. clavatum* spores a substance which they called "sporonin," to which they assigned the formula $(C_{10}H_{16}O_3)_x$. They considered the cell membrane to contain only about 2 per cent cellulose and 28.8 per cent sporonin.

Bowman (9) reported finding *Lycopodium* spores at a depth of 7 to 13 feet in the Kodiak peat bogs of Alaska. The spores of the Lepidodendreae—gigantic extinct relatives of *Lycopodium*—have persisted in coal (55, p. 237). R. Dodge (18) has reported the presence of silica in the spores of another Pteridophyte, *Isoetes Eatoni*.

The outermost layer of the spore coat of *Lycopodium* stains red with Sudan III, indicating the fatty or waxy nature of the cuticle or outermost membrane. Apparently this outer layer offers resistance to passage of water and to wetting, but it is soluble in alcohol.

The polarizing microscope shows a doubly refractive substance suggesting cellulose in the inner layer of the spore coat. This is confirmed by the sulphuric acid-iodine test (20, 23). The outer fatty and inner cellulose layers of the spore coats have apparently remained unchanged during the different periods of storage with various temperatures and humidities.

The middle layer, which is the thickest of the three layers, is netted in

all the species here studied except in *L. lucidulum*. This layer is silicious and doubtless adds greatly to the resistance of the spore coat to attacks of bacteria and fungi.

The *Lycopodium* spore coat is made up of three layers: (A) an outermost waxy cuticle; (B) a middle silicious layer; (C) an inner cellulose layer. It seems that the outermost waxy layer of the spore coat of *Lycopodium* renders it difficult to wet and resistant to changes in moisture, and the silicious layer resistant to fungi and bacteria. This must have a very direct bearing upon the difficulty of securing a high percentage of germination.

Resistance of Spore Coat to Bacteria and Fungi

The *Lycopodium* spore coat frequently has fungus spores and fragments of hyphae caught on, or in, the network of the raised pattern. Yet this spore wall is very impervious to bacteria and fungi, and although a culture may become overgrown with such organisms, the spore wall, except in rare cases, remains intact, probably due to the silicious layer. Careful watch was kept for any signs of erosion or destructive action on the spore coat by fungi or bacteria. Very seldom was either type of organism found to have penetrated a spore, and when this occurred, the spore coat appeared to have been cracked or broken by mechanical means, rather than broken down by enzyme action. Fungi have been found inside a few spores in very old cultures, such as the one of *L. obscurum* spores, which had been on sphagnum nearly 14 months, Nov. 20, 1933 to Jan. 14, 1935. Here a few spores were found to contain spherical fungus bodies, and in just one spore a fungus coil was found (see Fig. 3 A). It was not proved at what time the fungus entered, but observations would indicate that, in most cases, the fungus does not penetrate the spore coat.

Some attempts were made at sterilizing spores by J. K. Wilson's (70) calcium hypochlorite method, but this did not prove very successful or satisfactory. Damp peat or sphagnum have been found the best media for keeping fungus development at a minimum. Fungus is rather slow growing in Beyerinck solution, but especially rapid on potato dextrose agar or 2 per cent maltose.

MOISTURE CONTENT

Tests were made with spores of *L. complanatum* to find the effect on weight of dry and moist storage. Freshly-harvested spores were stored in open glass vials and placed, one in a dry desiccator over calcium chloride; the other in one in which distilled water had been substituted, thus making the humidity about 100 per cent. Spores stored over dry calcium chloride lost 8.7 per cent of their weight from Nov. 6, 1933 to Apr. 7, 1934. Those stored in the moist chamber gained 8.6 per cent of their original weight

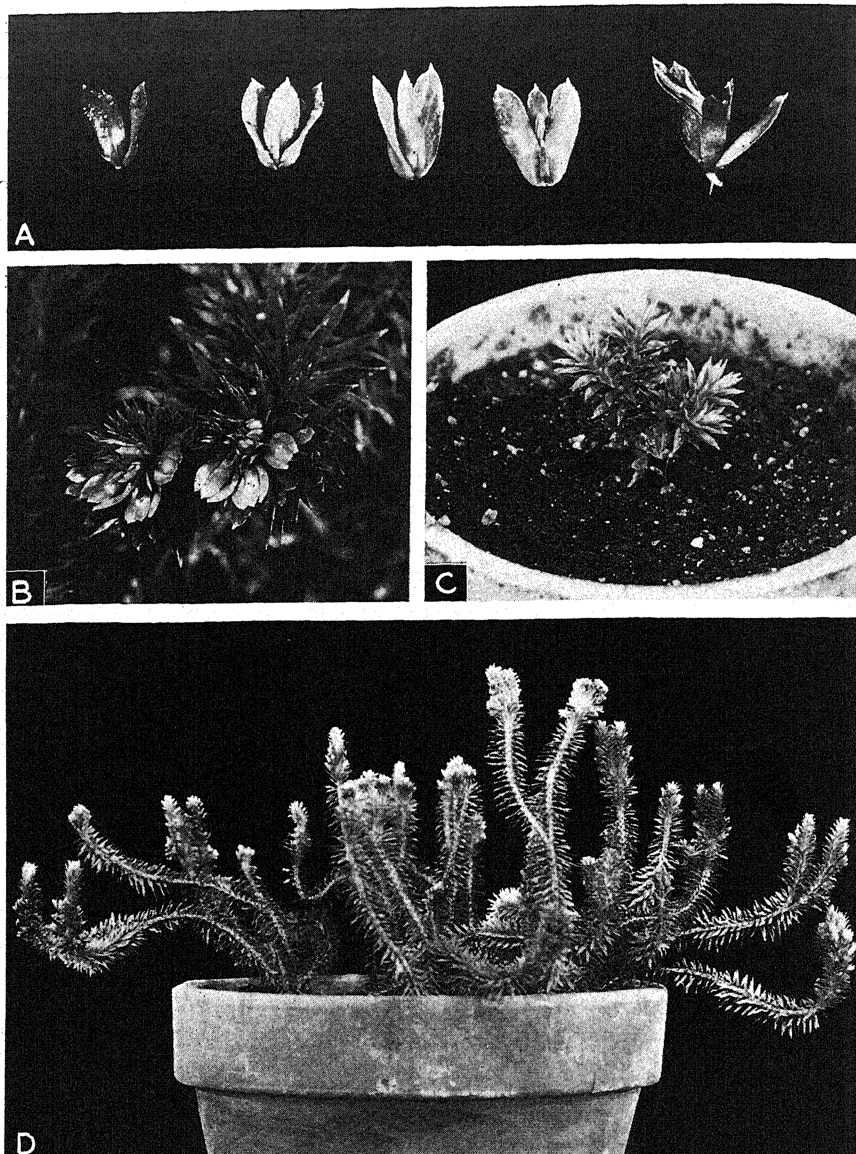

FIGURE 3. *Lycopodium lucidulum.* (A) Bulbils. × 2. At right, root and shoot development. (B) Bulbils on apical branches. × 2. (C) Bulbiling 10.5 months old. × 0.75. Transplanted twice to mixture of leaf mold, peat, and sand. (D) Collected plant grown in peat 1 year. Sprayed for mealy bug with trimethylene dithiocyanate 1:10,000 in late August. Apical growth stimulated. × 0.33.

in the same time. Loss of weight in the dry chamber was very rapid at first and 7.4 per cent occurred during the first 24 hours, and was nearly stable by the end of the first week. Spores in the moist chamber also showed the most rapid change during the first week, but continued to gain slightly in weight for a long period. These data seem to indicate that spores of *L. complanatum* can lose or gain water vapor through the spore coats, although they seem highly impermeable to liquid water.

The difficulty of wetting *Lycopodium* spores in water has long been known and is mentioned and discussed by numerous writers. This property led to their use as a dusting powder for infants and adults (8). In medicine, pills were often rolled in *Lycopodium* spores to keep them from sticking. It is interesting to note that the Japanese as well as Europeans employed *Lycopodium* for this purpose (37).

As early as 1857, Berg (6, 7) noted that the oil in the outer wall was soluble in alcohol or ether, and that spores after treatment would mix with water and sink. Lüstner (46) in 1898 considered the resistance to wetting of *Lycopodium* spores and its relation to their distribution and germination. Kessler (38), in 1914, discussed the effect of spore pattern and wetting on the ecological distribution of the species, and Fleischer (26), in 1929, the resistance to wetting of the netted type of *Lycopodium* spores. Dombrowski (19), in 1933, worked on distribution of spores of "Laubmoosen." Ziegenspeck (77), in 1934, has tested the wetting effect of a long series of substances, alcohols, acids, oils, etc., in different concentrations, upon the spores of *L. clavatum*.

OIL CONTENT OF SPORES

In freshly-ripened *Lycopodium* spores, a fairly large oil globule is usually present at the base of the spore. In practically all spores (except some kept frozen until March) the large oil globule usually disappears by mid-April. The oil content was studied most carefully in *L. complanatum*, since the largest quantities of these spores were available. Decrease in size of the globule was noted in January. The different temperature and humidity treatments seemed to have little differential effect—the globules seeming to disappear about the same time—a little more rapidly in the spores stored at higher temperatures than in those frozen or kept at 5° C. or below. Some small oil globules were still present up to mid-April, as shown by Sudan III, when the spores were crushed. But the total amount of oil seems to be less than in the fresh spores.

Some *Lycopodium* powder purchased from a druggist proved on microscopic examination to be spores of *L. clavatum*. A fat extraction by ether for three days, using the Soxhlet apparatus, yielded 8.5 per cent of odorless, yellowish oil. Microscopic examination and test of the extracted spores with Sudan III showed that most of the oil had been removed, al-

though some small traces remained. There was no way of determining the age or previous history of this sample.

For comparison extraction was made of a sample of *L. complanatum* spores which were collected Oct. 28, 1933, and stored in a cold ante-room until May 15, 1934. The Soxhlet was run six days on this sample and yielded 6.8 per cent oil. When the extracted spores were examined and treated with Sudan III, no oil was found on the surface of the spore coat. When crushed, the spores gave a strong fat test, showing considerable oil had been retained within the spore coat.

An extraction was made from *L. complanatum* spores of the following season's crop. These were collected Nov. 4, 1934, stored two weeks in the cold ante-room, and then left in a paper bag on the laboratory table until Jan. 10, 1935. Ninety-five per cent alcohol was used in the Soxhlet condenser for four days and yielded 7.5 per cent yellow oil. Microscopic examination of a sample revealed no visible oil globules, but when the spores were crushed, they gave a strong fat test with Sudan III. The remaining spores were further extracted with ether and yielded 2.95 per cent more oil, making a total extraction of 9.97 per cent of alcohol-ether soluble substances. Crushed spores again showed a strong oil test, indicating how resistant the unbroken spore coat is to the complete removal of the oil. As a check, a sample of the same collection of spores was run in ether alone and yielded 4.5 per cent oil. Sudan III on crushed spores showed that some oil had been retained. The oil was evidently more soluble in alcohol than in ether.

Various authorities differ in their statements in regard to the percentage of oil contained in *Lycopodium*. The lowest value found by early workers was 5 per cent [analysis by Bucholz, mentioned by De Candolle (14) in 1818] while "47% fixed oil, bland" was reported by Bentley and Trimen (5) in 1880. The method of analysis is not always stated, although Langer (43) extracted fresh *L. clavatum* spores with chloroform after grinding with quartz sand. He obtained 49.34 per cent. Oil from the older spores differed in showing more oxygen and rancidity. His analysis was checked fairly well by Rathje (49), who reported 49.2 per cent fat. The species was not mentioned here, but it was probably *L. clavatum*, as this seems to be the only species used extensively in pharmacy or analyzed according to the medical botanies. Most authors mention the inflammability of the spores, which gave the name of "vegetable sulphur" or "vegetable brimstone," and led to its use for fireworks, stage effects, etc.

VEGETATIVE PROPAGATION BY CUTTINGS AND BULBILS

CUTTINGS

The wide use of *Lycopodium* for Christmas decoration and its commercial exploitation has caused various groups interested in conservation

of native flora to realize the danger of extinction and to urge the use of less rare plants for Christmas greens. A few dealers in native plants list one or more species of living *Lycopodium* plants for sale. So far as it has been possible to determine, these plants are not propagated, but are gathered from the wild. It has seemed of interest in the cause of conservation to learn whether *Lycopodium* could be propagated successfully by vegetative means such as cuttings and bulbils.

Some preliminary experiments made with *L. complanatum* cuttings in the Barnard College greenhouse during the spring of 1933, resulted in about 30 per cent rooting and gave encouragement to undertake further tests. These cuttings were made Apr. 16, 1933. The results are summed up in Table IV. Tip cuttings of *L. lucidulum* made Sept. 20, 1933, produced roots in about two and a half months. By Dec. 7, 81 per cent had rooted in peat, and 89 per cent in peat and sand.

TABLE IV

CUTTINGS OF L. COMPLANATUM MADE APRIL 16, 1933, AND RECORDED MAY 31, 1933

Media	Total number	Number rooted	Per cent rooted
Sand	72	18	25
1 sand: 2 sphagnum	46	14	30
Sphagnum	212	75	35
Totals	330	107	32

TABLE V

LYCOPODIUM CUTTINGS

Species	Time in media	Media	Results
Greenhouse 1933			
L. comp.	Apr. 16 to May 31	Sand 1 sand: 2 sphagnum Sphagnum	25% rooted 30% rooted 35% rooted
L. comp.	Aug. or Sept. to Dec.	Sphagnum Peat Peat and sand Native soil	Very poor Very poor Very poor Very poor
L. clav.	Aug. or Sept. to Dec.	Same as above	Very poor
L. lucid.	Sept. 20 to Dec. 7	Peat Peat and sand	81% rooted 89% rooted
Cold frames with slat shade 1934			
L. comp. *L. clav.* *L. obscur.*	Apr. 30 to June 7 May 16 to Sept. 19 May 14 to Sept. 19	Peat and sand Peat and sand Peat and sand	New growth New growth New growth

Middle or basal cuttings of this species may root, or show extension of root growth already present, but eventually die and are incapable of producing new plants, when once the apical end of the stem is removed. Very short apical cuttings (under an inch) seldom root, although they may remain green for months. There was some mortality among the rooted cuttings after they were transplanted to a soil mixture of one part leaf mold, one part peat, and one part sand, but a good proportion made continued growth and about 50 per cent produced new bulbils.

A number of cuttings of different species were made at different seasons, and the most conclusive results are summed up in Table V.

Shade seemed to be an important factor, so light tests were started early in the work.

Light

Cloth of the following kinds was used on cold frames:

1. Muslin (cuts down light transmission about 50 per cent[1]).
2. Cheesecloth (cuts down light transmission about 35 per cent[1]).
3. Hospital gauze (cuts down light transmission about 20 per cent[1]).

The cloth was tacked on the inside of the sash just under the glass. Ventilation was given on mild days during the winter, and water as needed. Plants put in during the fall remained green and in good condition in spite of the severe winter of 1933–1934, and great extremes of temperature (twice down to $-20°$ F.). During many weeks the frames were covered with snow. No differences in the effect of varying shades were noted previous to the middle of April. The soil mixture was equal parts of peat, sand, and soil. The test was run until Oct. 10, 1934. The best survival was under the heaviest cloth, muslin, transmitting 50 per cent of light. The highest mortality was under hospital gauze, cutting down transmission of light about 20 per cent, and the cheesecloth was intermediate. The slat shade over sash has proved very satisfactory in the long run. Soil fertility, moisture, watering, and other environmental factors all played a part, but these were kept as nearly uniform as possible so that the light would be the only variable in these tests.

Transplanting Lycopodium

In the fall of 1933 six species of *Lycopodium* were removed from their native woods to the greenhouse to observe conditions for maintenance of normal healthy growing conditions. These results are shown in Table VI. These were grown in the orchid house under slat shade, with a fairly high temperature and humidity.

[1] Determined by J. M. Arthur.

TABLE VI

RESULTS OF TRANSPLANTING TESTS ON LYCOPODIUM

Species	Native soil	Peat	Peat and sand	Sphagnum
L. annot.	—	—	Fair	—
L. clav.	Fair	Fair	—	Fair
L. comp.	Fair	Fair	—	Fair
L. lucid.	Good	Good	Good	—
L. obscur.	Good	—	Good	—
L. trist.	—	—	Died	—

Young branches of *L. obscurum* in native soil made vigorous young growth beginning early in April. This species and also *L. annotinum* in peat and sand, started marked new shoot growth about the middle of April, and by the middle of May showed branching shoots up to three or four inches in height. *L. lucidulum* started the formation of new bulbils in January and made marked new apical growth.

In the spring, summer, and fall of 1934, collected plants of *Lycopodium* were transplanted to cold frames under sash with slat shade. Some were planted directly in the frames. Others were put into flats, which were easier to handle. Plants collected the last of April were put into flats of peat and sand to test rooting response. *L. clavatum* so treated made vigorous new apical growth up to seven to eight inches long during the summer, although some of the older upright woody branches died. On Oct. 31, these were transplanted from the flats into a cold frame containing leaf mold over a mixture of peat and sand and wintered well. In *L. complanatum* some of the oldest woody branches died, but new apical growth was produced by many of the vigorous young rhizomes. The growth in length was not as great as in *L. clavatum*, but a few young cones were produced. Plants put directly into the cold frame behaved in much the same way. The plants which had died by Oct. 31 were older branches and portions of the rhizomes lacking roots or active growing points. These became dried out and turned brown. The greatest loss was around the edges of the frames or flats where moisture was less. The ones in the center, where moisture was more uniform, maintained their green color for a longer period. Transplants of *L. obscurum* and *L. clavatum* were also made in the middle of May and were in good condition until the middle of September. Some plants of these two species moved in early June, when the new growth was young and succulent, were dead by September. *L. lucidulum* plants collected early in September were in excellent condition on Oct. 31, and wintered well. Some *L. obscurum* with fruiting cones were moved in the middle of September. These came through the winter in good condition and were shedding spores during March 1935.

BULBILS

The reproduction by bulbils of *L. lucidulum* has been reported in some detail by R. W. Smith (54) and he notes the annual periodicity and that two to four mature on an apex in September.

In transplanting cuttings and plants bearing bulbils, many snap off as they are very brittle. Results to date indicate that the early formation of bulbils in greenhouse cultures would be a fairly rapid means of propagation of *L. lucidulum*. In nature bulbils mature annually from late August through September. At least five or six months' time can be gained in the greenhouse, the first crop maturing in April, and the same plants bearing a second crop in September. The number produced seems to range from three to about nine, with an average around five or six (see Fig. 3 B). This average is considerably higher than that reported by Smith (54) for plants in the wild. Some of these bulbils which began to show in January, began maturing and dropping from the plants in mid-April. A few days after falling on the damp peat, they form a root and a shoot (definitely responsive to both gravity and light) which start into vigorous growth (Fig. 3 A).

Bulbils planted Sept. 20, 1933 grew into sturdy young plants, some of which soon showed the typical dichotomous branching (see Fig. 3 C). Figure 4 C shows bulbilings in the pot at the right as compared with rooted cuttings of the same age in the flat at the left (Fig. 3 B). Care needs to be used in watering young plants to prevent washing out. Petri plates were used over and under the pots for about a month to allow watering from below until the roots were well anchored. Even after transplanting, too much force in watering tends to wash out the young plants. The bulbilings are very responsive to light, especially from one side.

Important Factors in Vegetative Propagation

In the literature, there are a few suggestions on handling *Lycopodium*. Nearing (48, p. 97) gives some cultural hints for *L. lucidulum*, describing its habitat as "in dark corners of very old woods" and finds it intolerant of sunlight or drying winds. He says, "You can transplant this moss if you have a place rich in woods leaf mold, and protected from sun and wind. It will also survive a long time in a house if placed under a glass to preserve a moist atmosphere." He also finds the transplanting of sprouting bulbils "an easy matter."

Roberts and Lawrence (28, 52, p. 314) in a note in the September, 1933 Horticulture have recommended *L. lucidulum* for rock gardens and report 98 per cent rooting from 50 three-inch cuttings in peat and sand. Their cuttings made larger, better plants than bulbils.

The writer's tests have shown that *L. lucidulum* is one of the easiest

species to transplant, probably because, from the nature of its growth and habitat, it is easier to retain more of the root system. The cuttings of this species root more readily than other species, and it also has the advantage of propagation by bulbils. As previously noted, adult plants kept in the greenhouse can produce two crops of bulbils annually, as compared with one in nature. Rooted cuttings also produce bulbils the first season.

Roberts and Herty (51) have shown that it is useless to transplant old lignified plants of *L. complanatum* var. *flabelliforme* unless the whole root system of the connecting portion is attached, and that the best results come from using young vigorous apical growth.

The writer's tests support these findings and also add four more species to the list. It was found possible to transplant *L. annotinum*, *L. clavatum*, *L. complanatum* var. *flabelliforme*, *L. lucidulum*, *L. obscurum*, and maintain them in healthy condition in either greenhouse or cold frame during the winter months. Plants were also successfully carried through the summer months in slat-shaded cold frames. *L. tristachyum* Pursh. which has very deep rhizomes, almost impossible to secure intact, proved very difficult to transplant.

In shipping or moving *Lycopodium*, it should be kept from drying out. Good results were obtained by packing immediately in live damp sphagnum moss, and then wrapping in several layers of newspapers. Handled in this way it can be kept in good condition for shipment for considerable distances, or can be stored in a vasculum for several weeks. Low temperatures or storing in a cool place also facilitates keeping qualities.

Water relations. Either excessive moisture or excessive dryness seems to be injurious to *Lycopodium*. Some species like *L. lucidulum* normally grow in wetter habitats than others. This species is commonly found in black mucky swamps or along edges of brooks but usually where water is changing and not stagnant. Standing water causes damping off not only of this species but of others tested. Hence drainage seems essential. If kept too wet, various fungi develop quickly and may be decidedly injurious. On the other hand too little moisture causes drying out, and may also result in the death of the plants.

Light. Some of the most luxuriant natural stands of *Lycopodium* are found on north slopes and in hemlock ravines, which are well shaded and have a good supply of moisture, but are well drained. Some protection from full sunlight seems essential. The only species found in more exposed situations is *L. complanatum*, which sometimes grows in the edges of old fields. There the color is usually yellowish, and the growth much less luxuriant than in the deeper shade in the woods. For artificial culture, slat shade over cold frames or the equivalent of 50 per cent normal sunlight gave best results.

Soil acidity. There seemed to be little record of the normal acidity of

Lycopodium stations, so the acidity was tested throughout the growing season. The first soil samples were collected March 29 to 30, 1934, from eight stations of *Lycopodium* and *Epigaea repens* L. in northern Connecticut. The ground was still frozen to within an inch or two of the surface, especially in the woods on north slopes. The samples were collected in glass bottles and vials and carried in a vasculum to the Institute, where they were stored in a cold dark room with temperature just above 0° C. until determinations could be made. These were made by a Youden quinhydrone hydrogen ion concentration outfit (73). The pH ranged from 7.27 to 8.07 on the March samples.

Another set of nine soil samples was collected in the same region and some at the same stations on Apr. 28, 1934. It was then possible to get deeper samples around the roots of the plants. In most cases a layer of black humus and leaf mold extended from an inch to several inches below the surface. One station of *L. clavatum* and *Epigaea repens* had a thin layer of leaf mold over a yellowish sandy soil. The range in these samples was pH 6.97 to 7.23.

The tests throughout the growing season are summed up in Table VII.

TABLE VII

pH OF LYCOPODIUM STATIONS 1934

Date	No. stations	Range for 6 species	*L. comp.*	*L. obscur.*
Mar. 29–30	8	7.27–8.07	7.79	7.67
Apr. 28	9	6.97–7.23	7.08	7.06
May 28–29	18	6.67–7.21	6.97	6.83
July 29	19	6.52–7.02	6.93	6.87
Sept. 2–3	13	5.42–7.09	6.52	6.68
Oct. 1	6	6.35–6.85	6.63	6.55
Nov. 4	4	5.30–7.11	6.14	6.30
Nov. 25	9	5.28–6.85	5.42	5.28

Eighty-six samples, all from northeastern Connecticut, were tested and the range for the whole season was from pH 8.07 to 5.28. The last two columns give the range on two species, *L. complanatum* from 7.79 to 5.42 and *L. obscurum* from 7.67 to 5.28. Less complete tests were made on the other species, but the general trend was the same, and toward increasing acidity, so that the late fall samples were decidedly more acid than the early spring samples.

Voss and Ziegenspeck (67) mention a pH of 2.42 around the roots of *L. annotinum* and Asai (1) a pH of 5.66 on June 24th for *L. clavatum*.

A soil mixture of peat, sand, leaf mold, and soil proved fairly satisfactory. The soil problem needs further investigation.

Pests. In the greenhouse *L. lucidulum* (and *L. clavatum* to a less degree) became infested with mealy bug. Some of the synthetic insecticides of

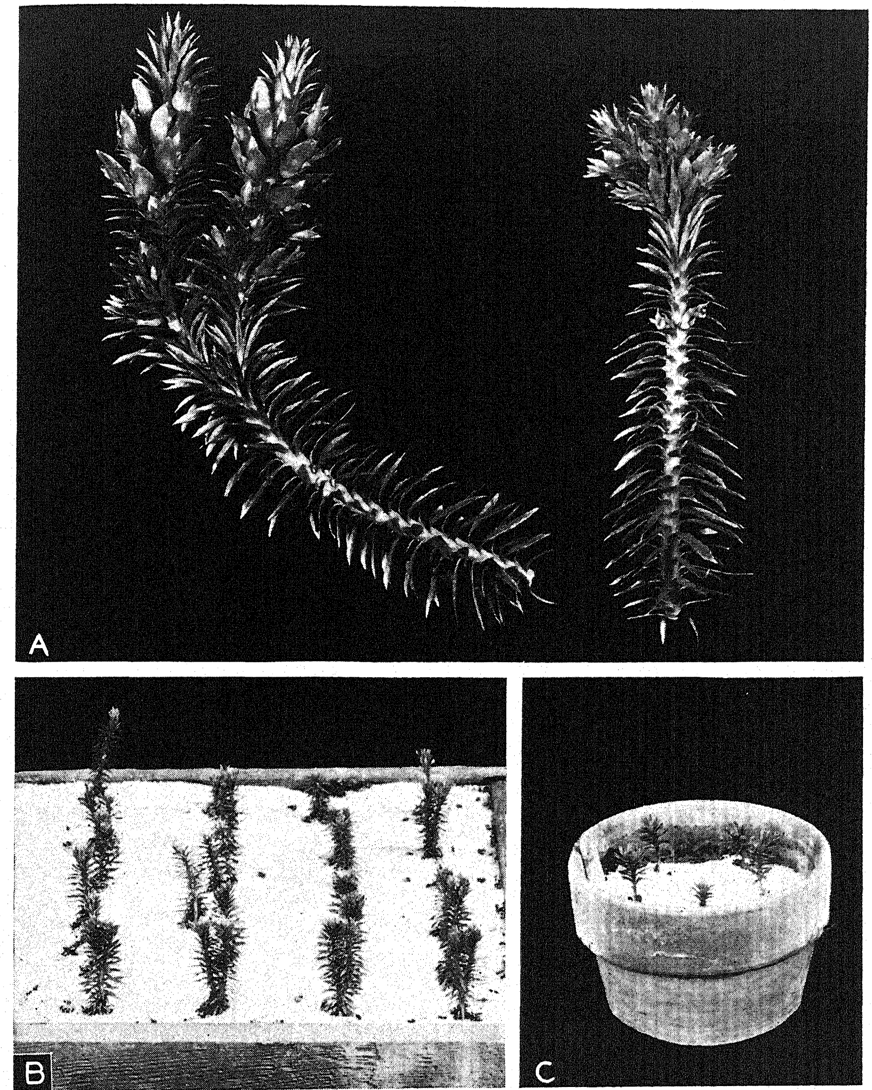

FIGURE 4. *L. lucidulum.* (A) Left, branch from plant sprayed with trimethylene dithiocyanate last week of Aug. 1934. Right, control not sprayed. Photographed Sept. 10, 1934. Difference in length of new apical growth and development of bulbils. × 1. (B and C) Cultures aged 6 months. Grown in mixture of leaf mold, peat, and sand. × 0.25. (B) Cuttings rooted in peat. (C) Bulbilings rooted in sphagnum.

Hartzell and Wilcoxon (29, 69) were used as a spray in dilutions of 1 to 10,000. These killed nearly all the mealy bugs, and the only injury to the plants was a slight browning on the edges of the leaves of the young growing tip, not all tips being affected. Trimethylene dithiocyanate, used the last of August, not only killed most of the mealy bugs, but seemed to have a stimulating effect on new growth. The new apical growth with bulbils was already initiated; but within ten days to two weeks after treatment the sprayed plants showed greatly increased growth over the non-sprayed controls. This stimulation could not have been due entirely to the killing of the mealy bugs, for not all tips were infested, but all apical growth was markedly increased (Figs. 3 D and 4 A).

Sowbugs, slugs, and earthworms may at times be numerous and become troublesome.

SUMMARY

1. Spores of *L. complanatum* var. *flabelliforme* Fernald, which had been stored for approximately five months, Sept. 30, 1933 to Feb. 22, 1934, at constant temperatures ranging from 1° to 35° C. and one alternation from 5° to 25° C., germinated on peat after a year of incubation at room temperature. The percentage of germination ranged from 1.1 to 16.3 per cent. *L. obscurum* L. spores placed in humidity chambers ranging from dry (over $CaCl_2$ or concentrated H_2SO_4) to 90 per cent of humidity for two weeks, in Nov. 1933, gave germination on damp sphagnum at room temperature after about 14 months, Nov. 20, 1933, to Jan. 14, 1935. *L. obscurum* L. spores washed in 95 per cent alcohol for two minutes germinated on several culture media, i.e., 2 per cent maltose, sphagnum, peat and soil in about one month, Nov. 8 to Dec. 5, 1934. A few *L. annotinum* spores treated in the same way also germinated.

2. The spore coat of *Lycopodium* is made up of three layers: (A) an outermost waxy cuticle; (B) a middle silicious layer; (C) an inner cellulose layer. This structure renders it highly resistant to wetting and also to attacks of bacteria and fungi.

3. *Lycopodium* may be propagated vegetatively by cuttings and bulbils. Spring cuttings of *L. clavatum* L., *L. complanatum* var. *flabelliforme* Fernald, and *L. obscurum* L. did better than fall cuttings. Fall cuttings of *L. lucidulum* Michx. rooted well.

4. *L. lucidulum* Michx. can be propagated by bulbils, two crops of which can be produced annually under greenhouse conditions. Some rooted cuttings will produce bulbils the first year.

5. Only the young apical growth of rhizomes makes satisfactory cuttings. Lignified tissue usually dries out and dies.

6. Transplanting tests have shown that it is possible to maintain *L. annotinum* L., *L. clavatum* L., *L. complanatum* var. *flabelliforme* Fernald,

L. lucidulum Michx., and *L. obscurum* L. for considerable periods in either shaded greenhouses or cold frames. It is useless to move mature woody plants unless the root system is kept intact. Best results are obtained by using only young actively growing rhizomes.

7. Important factors in vegetative propagation are water relations, light, soil acidity, and pests.

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PROPAGATION OF LYCOPODIUM. II. ENDOPHYTIC FUNGUS IN GAMETOPHYTE AND SPOROPHYTE

FLORENCE L. BARROWS

Endophytic fungi have been known in plants for some time, and the more recent work by Melin (22) on forest trees; Freisleben's (7) experiments on Ericaceae and (8) on mycorrhiza of *Vaccinium;* the isolation of pure cultures by Hatch (11, 12, 13); and the work of many others (6, 9, 19), have drawn attention to the wide distribution of this type of fungus in the higher plants. Pteridophytes are also known to have endophytic fungi.

As indicated in the last column of Table II, Part I (2, p. 270) endophytic fungi in the prothallia of various species of *Lycopodium* have been reported by several workers, beginning with Treub in 1884. Treub (30) germinated *L. cernuum* L. spores on soil from native stations and concluded that it must have a fungus to develop. He thought the fungus was "probably *Pythium.*"

Lang (21) noted an endophytic fungus mycelium in the prothallia of *L. clavatum* L., and reported it non-septate with multinucleate vesicles similar to those recorded by Janse (18).

Probably Bruchmann (3, 4), with his work on *L. annotinum* L., *L. clavatum* L., *L. complanatum* L., and *L. Selago;* and Holloway (14, 15, 16, 17), for the New Zealand species, have made as thorough a study as anyone on the fungus in the gametophyte or prothallial stage. Bruchmann found that the germinating *Lycopodium* spores were independent of fungus up to the 5-celled prothallium; but thereafter were dependent during their life history upon a "Pilzgenossen." The fungus is found in the cortical tissue in *L. clavatum*, is intracellular in the outer, and intercellular in the inner layers; while in *L. Selago*, he found it to be only intracellular. The endophytes differ in the two types of prothallia and in *L. Selago* he reports "sporangioles." He thinks the function of the fungus is associated with excellent nutrition and produces a rich storage of starch.

Spessard's figures (27, p. 75) clearly show fungus in the prothallia of *L. clavatum* and *L. complanatum.* In his later report of 1922 (28) on prothallia of *L. lucidulum* and *L. obscurum* var. *dendroideum*, he says (p. 412), "The endophytic fungus is probably not *Pythium.* The reproductive structures point to this genus, but the vegetative and certain doubtful structures point to the Ascomycetes." "The habit of growth and the appearance of the reproductive structures indicate that the same species of fungus is not present in the two prothallia."

The literature contains no record of the isolation of fungi from *Lycopodium* gametophytes, but this was accomplished by William H. Davis[1]

[1] Personal communication.

of Massachusetts State College, who took the photos to illustrate Degener's paper of 1924 (5) on the discovery of numerous prothallia of *L. obscurum* at Amherst in May and June 1922. At that time Prof. Davis isolated a fungus but has never published his results. He was kind enough to describe his methods to the writer.

The Degener station at Amherst (5) and the Stokey and Starr (29) stations near Mount Holyoke were visited in Oct. 1933, but no gametophytes or sporelings were found.

L. C. Petry of Cornell furnished two lots of *L. obscurum* gametophytes, received on Aug. 17, 1933, and Aug. 29, 1934. The first lot consisted of two gametophytes with sporophytes attached, and 18 gametophytes. The plants of this lot were kept in a north room in a glass container, and partly covered with a glass plate, for about two weeks. Several days of very damp rainy weather with high humidity occurred during the last of August. Some gray-green mold appeared on the surface of the soil, also on one or two prothallia, and even on the tip of one sporeling, on one of the dichotomous branches which had been slightly bent and injured. Unfortunately, at the time the fungus was not carefully examined or recognized as being possibly significant. No laboratory facilities were available for isolation. These sporelings and gametophytes were transferred to Boyce Thompson Institute on Sept. 1, and were maintained in a moist chamber in a living condition for several months. A few were removed for study. One small prothallium was preserved in formal-alcohol, Dec. 15, 1933, imbedded in paraffin, sectioned and stained, and found to contain the typical fungus, and also showed antheridia. No archegonia were found in this material, but several were found in May, 1935 (see Fig. 2 D and E).

ISOLATION OF FUNGUS FROM L. OBSCURUM GAMETOPHYTE

On Jan. 24, 1934, a larger prothallus, which had made recent extensive growth, was divided. Part was preserved in formal-alcohol for imbedding. The other part was washed in several tubes of sterile distilled water, and sectioned under aseptic conditions, and the sections planted in sterile distilled water, Beyerinck solution, 2 per cent maltose, potato dextrose agar slants, and potato dextrose agar plates. Fungi grew in nearly all these cultures. Sub-cultures have been made and carried along to the present.

Stained slides from the imbedded half of this gametophyte showed the fungus coils very clearly (as did the living tissue). The fungus stains red with Flemming's triple stain. This prothallus was producing abundant antheridia, but no archegonia.

NATURE OF THE FUNGUS IN PURE CULTURE

On solid media. From most of the cultured sections of prothallium there developed an aerial mycelium which was hyaline at first, but later became

darker, sometimes being faintly pinkish before it started to fruit, when it became gray-green. As the cultures aged and especially as they dried somewhat, they became much darker, passing through olive shades to a taupe or brown in very old or dry cultures. Transfers from these dark brown cultures give again a cottony-white mycelium which soon develops the gray-green color. The fungus may remain viable for months in these dried cultures and starts new growth when transferred to fresh media. On potato dextrose agar, in either plates or flasks, the fungus forms a spreading white mycelium with radiating lines. In about five days a definite ring is formed, usually quite green in color, and with a fainter outer ring. In

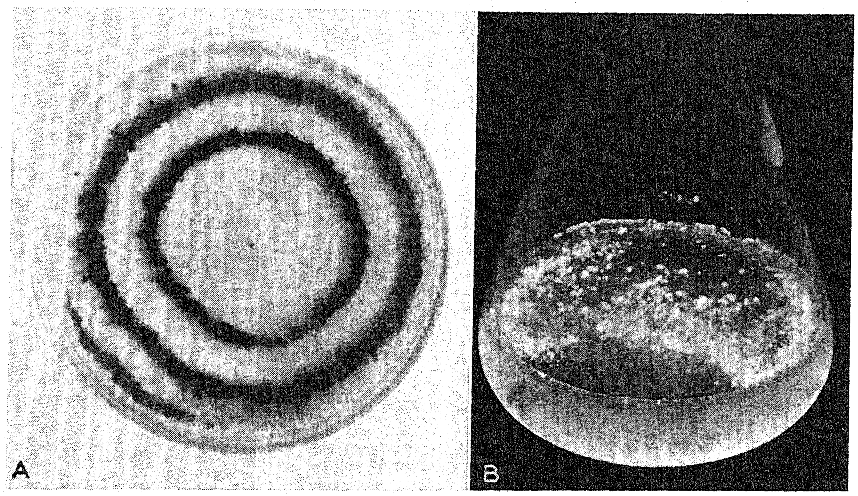

FIGURE 1. Pure cultures of fungus isolated from *L. obscurum* gametophyte. (A) Twelve-day growth on potato dextrose agar plate. ×0.57. (B) Five-day growth on 2 per cent maltose. ×0.53.

a week both rings are usually green. Figure 1 A shows the beginning of a 3rd ring after 12 days. Shortly afterwards the whole surface may become overgrown and entirely green. On standing it passes through the darker color changes just described. On potato dextrose agar slants the green color usually appears first at the upper drier edge of the slant and gradually extends to the base. Microscopic examination shows a very large number of hyaline to greenish conidia borne on typical flask-shaped branches.

On liquid media. Cultures in liquid media, such as distilled water, Beyerinck solution, and 2 per cent maltose (Fig. 1 B), form a white pellicle at the surface, or develop a submerged mycelium, which contains spherical bodies, especially as it grows older. These may also be found on agar, either at the base of the slant, or in the horizontal mycelium on plates, i.e., the

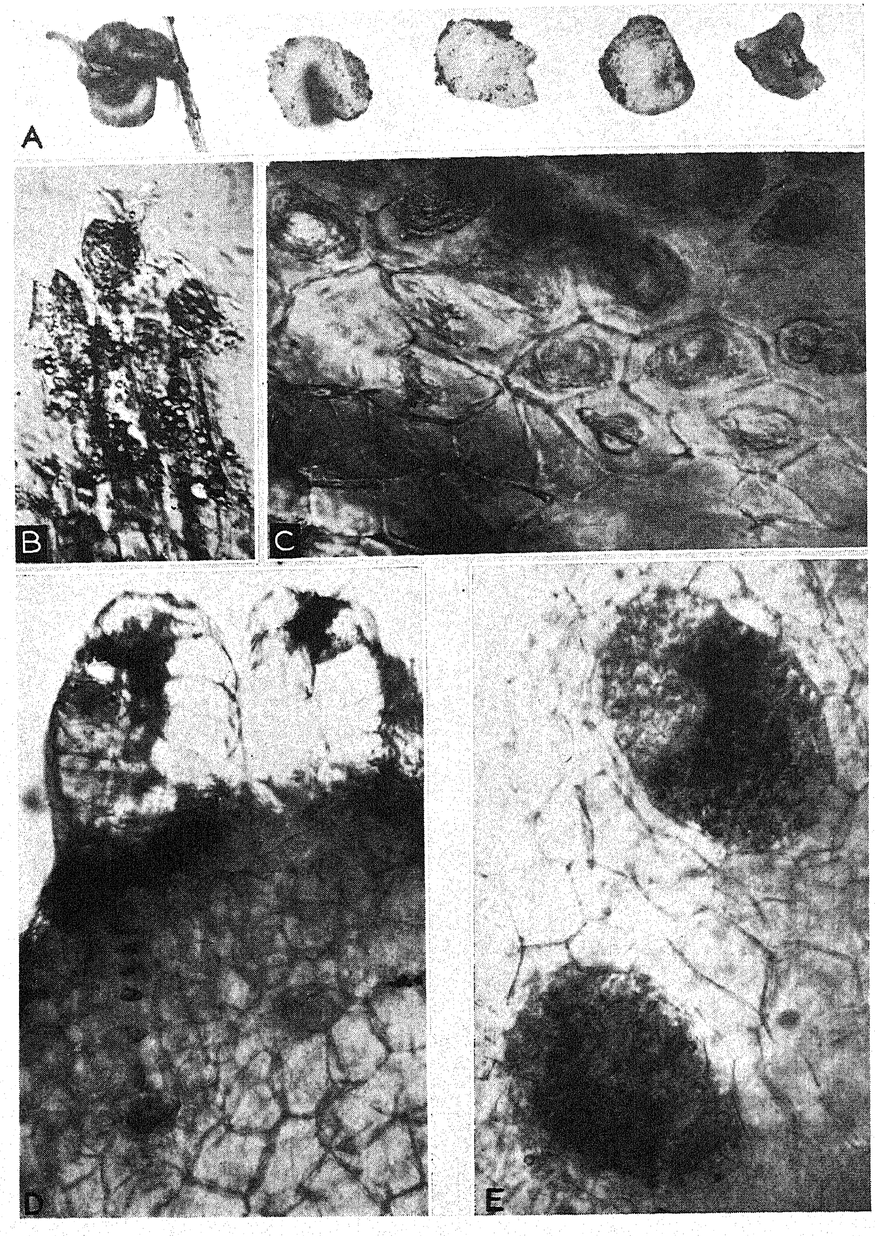

FIGURE 2. *L. obscurum* gametophytes. (A) ×1.5. (B) Oil and endophytic fungus coils in × sec. (C) Fungus coils. ×1120. (D) Archegonium with egg and canal cells. long. sec. (E) Antheridia. ×320. No fungus around gametes.

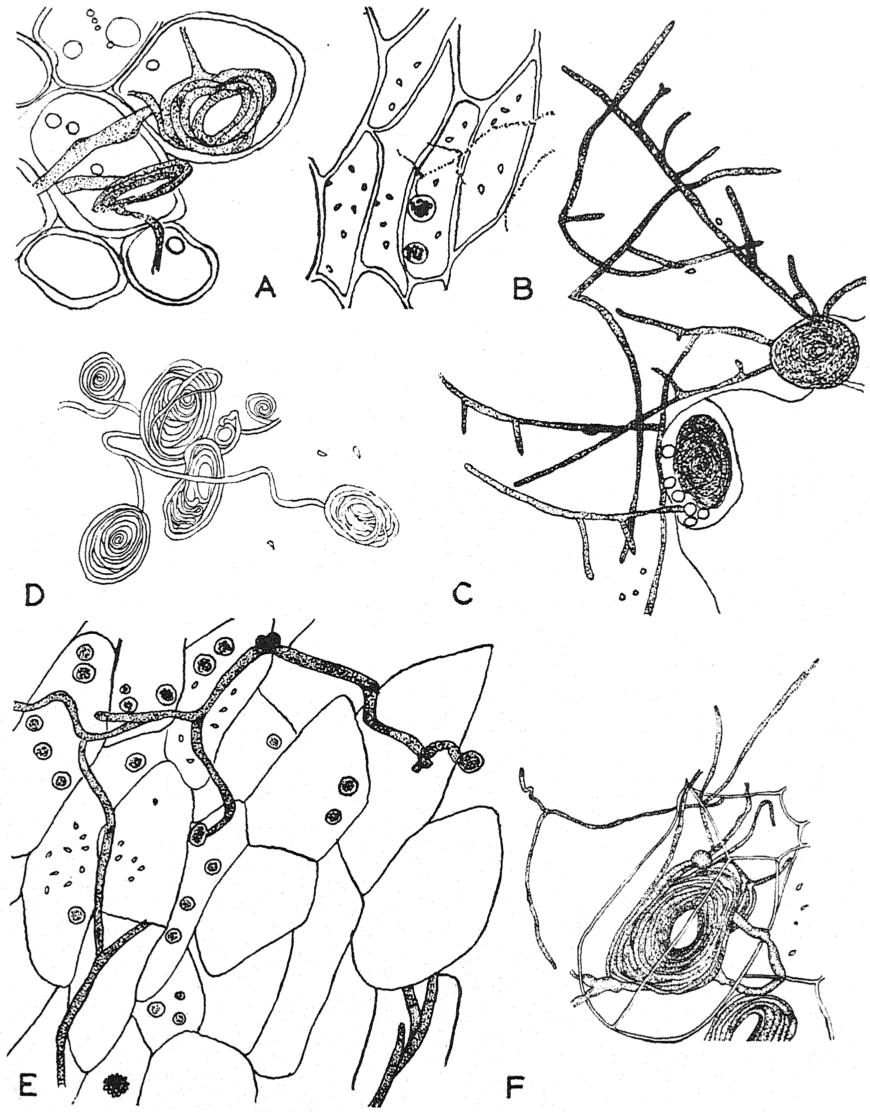

FIGURE 3. Fungus in *L. obscurum* gametophytes, drawn with aid of drawing ocular. (A) Oil globules and coils. (C) Same gametophyte with fungus grown out from coils in 2 per cent maltose in 2 days. (B and E) Spherical fungus bodies and small motile cells in gametophyte. ×290. (D) Coils separated from gametophyte. ×500. (F) Hyphae grown out from coils on slide in water over night. ×538.

moister regions. These spherical bodies suggest the multinucleate vesicles of Janse (18), and the "sporangioles," "sphaeromen," and "Chytridien" of Bruchmann (4), which have been reported in the gametophyte stages of *Lycopodium*. When material containing these spherical bodies is transferred to solid media, in a few days mycelium with greenish conidial branches is formed. If the greenish conidial stage is transferred back to liquid media, the spherical bodies are again produced.

ENDOPHYTIC FUNGUS IN GAMETOPHYTE

Other isolations from *L. obscurum* gametophytes have been made. The second lot of gametophytes received on Aug. 29, 1934 (Fig. 2 A, B, C) were used for isolations on Aug. 31, 1934, and again Feb. 11, 1935. These have given the typical fungus, with conidial stages greenish and also with the spherical or slightly elongate bodies producing tiny motile cells (Fig. 4).

In culture it was possible to see the hyphae with their typical right-angled branches growing out from the coils in the cells of the cut prothallus (Fig. 3 C). In the Feb. 11th sections, oil drops (tested with Sudan III) were numerous in the prothallial cells. In cells which were well filled with fungus coils, the oil drops were fewer and smaller, or had sometimes completely disappeared (Fig. 3 A).

On April 25, 1934, one of the *L. obscurum* sporelings was observed to be rather yellow and unhealthy-looking. Investigation showed that the gametophyte was disintegrating, as well as other gametophytes not bearing sporophytes. Live nematodes were present. The prothallia had lost their firm solid texture and had become dark brown empty shells, so light that they floated in water. On microscopic examination many of the remaining cells were found to be empty except for tiny cells resembling the contents of "vésicles" or "sporangioles" (Fig. 5 A and B). In most cases these were floating free inside the *Lycopodium* cells. Hyphae were also present, bearing a few of the spherical bodies, and there were numerous spherical bodies lying free and unattached to hyphae (Fig. 3 B and E).

Some of the smallest cells, which were apparently escapes from the spherical bodies, were motile and swimming free. In fact, empty spheres were found. Many of the hyphae were non-septate, but especially in the outer layers of the prothallus, or sometimes in the branches bearing the spheres, cross-walls were found. The cells of the prothallia were practically empty, the contents having disappeared (Fig. 5 A and B).

From these disintegrating prothallia cultures were made and on potato dextrose agar produced again the typical greenish fungus. These have been sub-cultured and carried along to the present and seem to be the same fungus as that originally isolated. Similar disintegrating prothallia were again found in March 1935, with dense coils of hyphae (Fig. 3 D and F), numerous spherical bodies, and small motile cells.

Further study of the isolated fungi confirms the conclusion that the green conidial stages are produced in the drier portions. However, floating mycelium on liquid media, such as 2 per cent maltose, may produce the conidia in huge numbers (Fig. 1 C). Conidia seem to be produced only on aerial portions of the mycelium. Some germinating conidia have been found developing hyphae (Fig. 4 C). The conidial branches may be either septate or non-septate (Fig. 4 A and C).

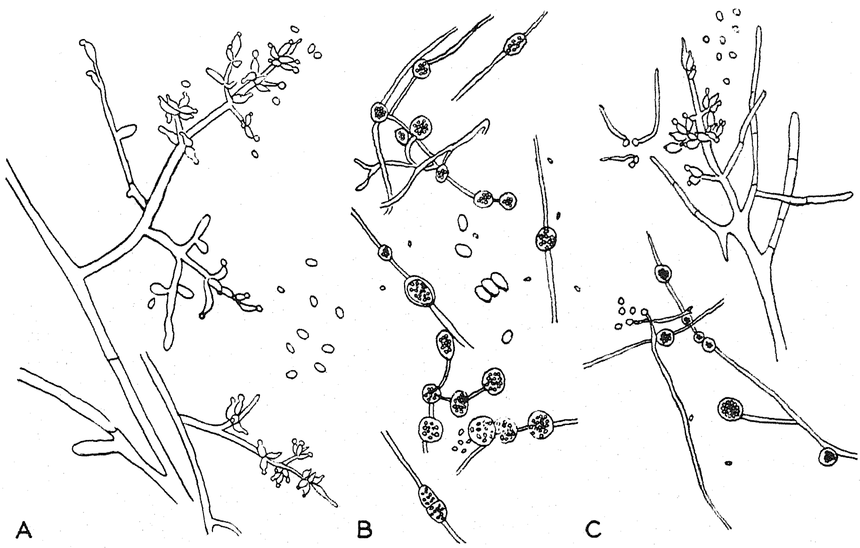

FIGURE 4. Pure cultures of fungus isolated from *L. obscurum* gametophytes. ×233. (A and B) On potato dextrose agar. (A) Conidia on aerial branches. (B) Spherical bodies and small motile cells. (C) Germinating conidia on 2 per cent maltose.

The spherical bodies are produced in the moister regions, and seem to be reproductive organs. They may be terminal on hyphae; or interposed in the hyphae, intercalary, the latter suggesting the position of oögonia of *Oedogonium*. At first the contents are granular, but later become divided off into separate tiny cells, varying in number, but with a definite orientation. They are somewhat pear-shaped, or shaped like a Prince Rupert drop. The larger spherical end is oriented toward the outside of the sphere, and the tapering end is toward the center (Fig. 5 A and B). [See also Part I, Fig. 1 B, Center (2, p. 274).] Many times the contents of these spheres have been seen in violent motion. Eventually the enclosing spherical wall breaks and the tiny motile cells are set free. They swim about freely in water and remain active for some time. Apparently they have flagella or cilia, or some swimming organ, in addition to being flexible, but it is difficult to see. Two often seem to be attached or entangled and jerk violently

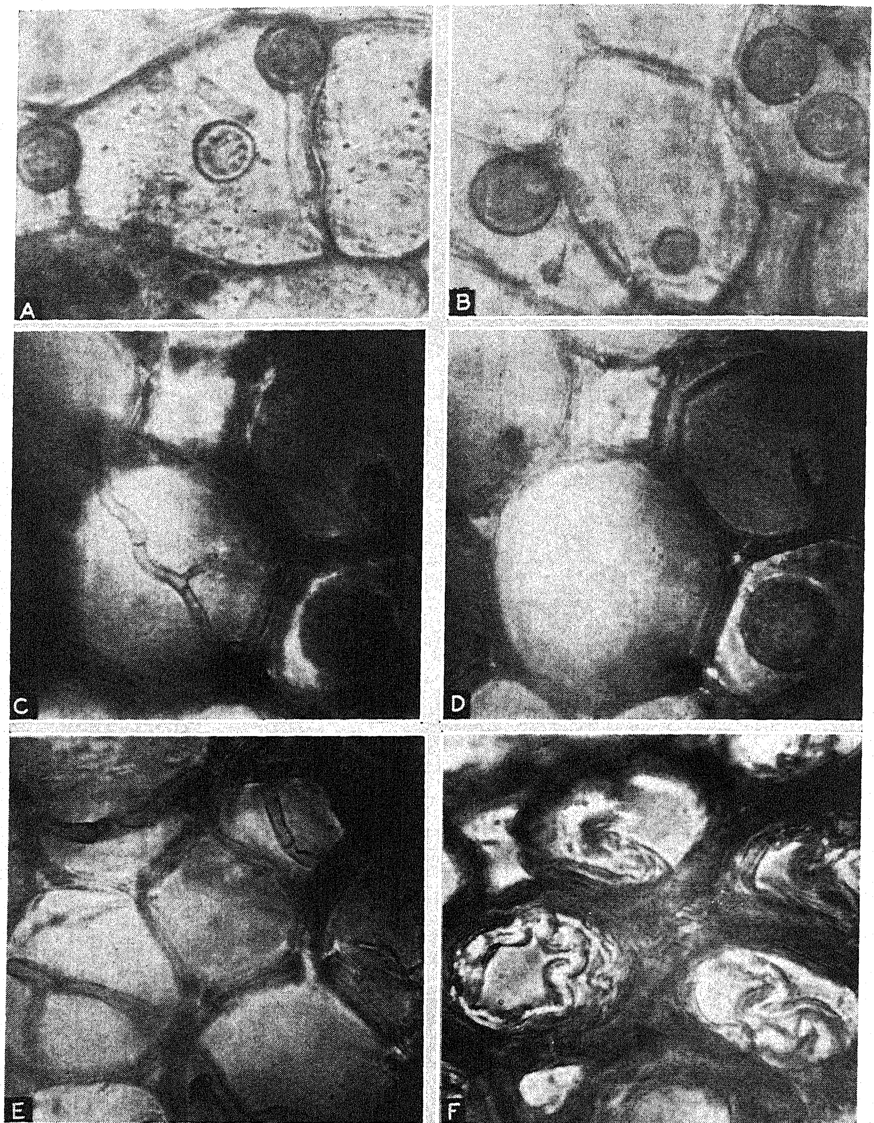

FIGURE 5. Endophytic fungi in *Lycopodium* gametophytes and sporophytes. ×644. (A and B) *L. obscurum* gametophyte with spherical fungus bodies and small motile cells. (C, D, and E) *L. obscurum* sporophyte. Rhizome with endophytic fungus in cortex. (C and D) Different foci of same cells to show septate hyphae and spherical bodies. (F) *L. lucidulum* sporophyte with endophyte coiled in cortical cells near active roots.

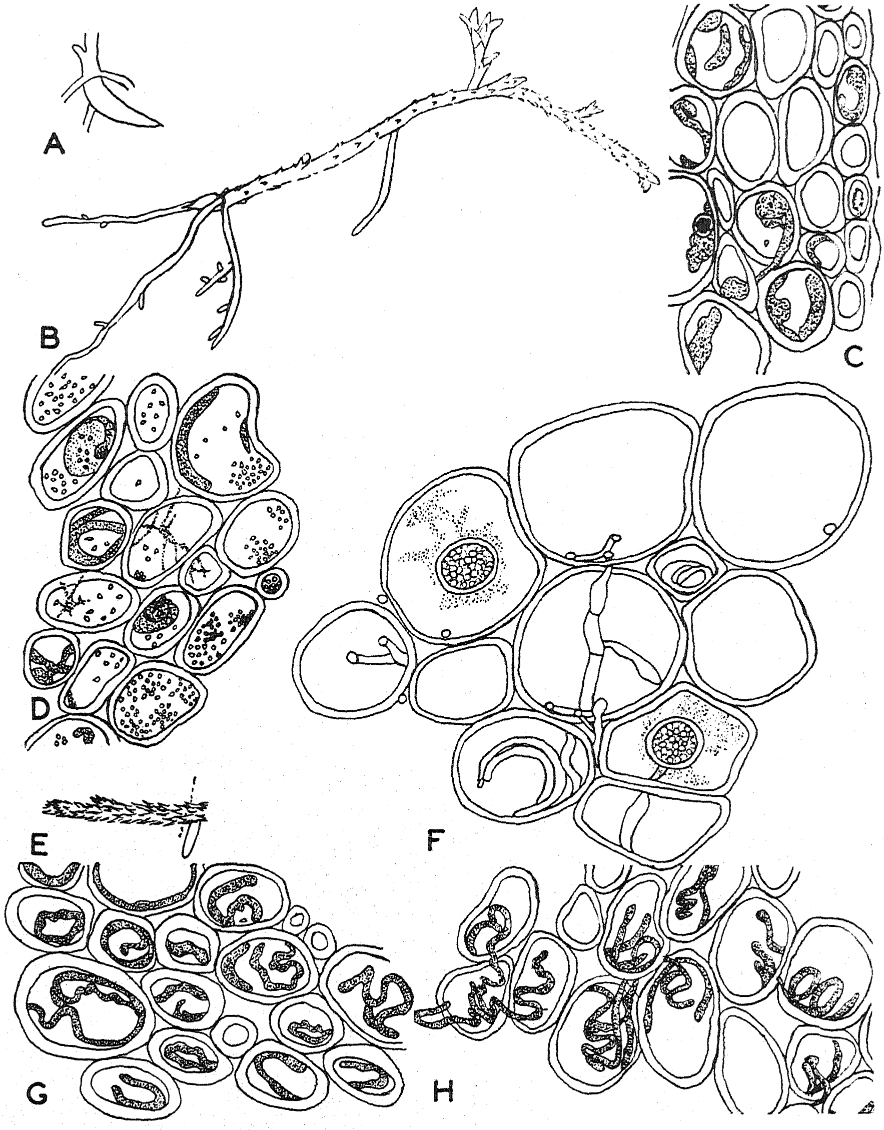

FIGURE 6. (A and B) *L. complanatum* sporeling. (A) ×1.42. (B) ×0.71. (C to H) Endophytic fungus in sporophytes. (C) *L. complanatum* ×sec. rhizome, fungus in cortex 1 inch from apex. ×280. (D) *L. clavatum* xylem with hyphae and motile cells. ×280. (E) End of *L. clavatum* rhizome where section (D) was made. ×0.67. (F) *L. obscurum* older rhizome. Same cells as Figure 5 (C and D). ×386. (G and H) *L. lucidulum.* (G) Coils in cortex, Nov. (H) Cortex, coils, Apr. ×280.

until separated. On addition of strong I-KI solution, the motion is quickly stopped. If very dilute solution is used they may retain their motility for some time. In dilute solutions of toluidine blue (0.1 per cent or less) they sometimes show activity for two days. On old or dried cultures, the walls of the spheres may become much thickened, and may change from hyaline or transparent to a dark brown.

It is hoped that further work will permit identification of the fungus. From present work it is still impossible to state whether this fungus is the one always found present in gametophytes of *L. obscurum*, or whether several species or genera may be capable of forming a symbiotic relationship as in *Pinus* (22).

So far it has been impossible to secure any quantity of gametophytes of other species of *Lycopodium* for isolation tests. The one *L. complanatum* var. *flabelliforme* Fernald sporeling found Aug. 29, 1933, had a prothallus attached (Fig. 6 A and B). This plant was grown in peat from Sept. 18, 1933, but, in an attempt to prevent drying out, was kept too wet. By Dec. 20, it was in a dying condition, and had developed a green fungus similar in gross appearance to the fungus isolated from *L. obscurum* gametophytes.

ENDOPHYTIC FUNGUS IN THE SPOROPHYTE

Living rhizomes of the sporophytes of *L. clavatum* L., *L. complanatum* var. *flabelliforme* Fernald, *L. lucidulum* Michx., *L. obscurum* L., and *L. tristachyum* Pursh., collected during September and October 1933, were examined in fresh hand sections, and all found to contain living fungi. So far as the writer has been able to determine, the literature contains no record previous to this date of the presence of fungi in the sporophytes of these species, although Roberts and Herty (24) in 1934 raise the question of the possibility of an associated fungus in the native soil necessary for the life of *L. complanatum* var. *flabelliforme* Fernald, and Voss and Ziegenspeck (35) in 1929 mention and figure *L. clavatum* and *L. complanatum* as having nodules on their roots. Asai (1) in 1934 lists *L. clavatum* as containing an endophyte on June 24th, but gives no figures or description.

Rayner (23, p. 178), in her discussion of mycorrhiza, says, "The sporophyte of *Lycopodium* has commonly been described as free from fungus infection and there is at present no record of the formation of ordinary mycorrhiza by any species. In view of the existence of what is probably an obligate relation in the gametophyte phase, the subject is of some interest, and there are observations of possible significance in the literature."

In this connection she mentions:

1. Bruchmann (1874) finding a tuberous development in the young stem of *L. inundatum*—"Polstergewebe"—which he believed to function in relation to storage of water.

2. Kühn (20) made similar observations on *L. inundatum* and noted a fungus with intracellular hyphae.
3. Goebel (10) also reported fungus in the sporophyte of this species.
4. Treub (30, 31, 32, 33, 34) observed mycelium in the young plant of *L. cernuum*.

The study of fungus in living *Lycopodium* sporophytes was continued in the spring, summer, and fall of 1934. Specimens of the first four species listed (*L. clavatum*, *L. complanatum* var. *flabelliforme*, *L. lucidulum*, and *L. obscurum*) were collected in northern Connecticut, March 30, as soon as frost permitted, and again Apr. 22, May 27 to 30, July 29, Aug. 19, Sept. 2, Oct. 1 and 13, and Nov. 4 and 25, the first snow having fallen on Oct. 13, and several hard freezes occurring before the last collection in November. The active growing season was thus thoroughly covered. *L. annotinum* was obtained from the Yale Forest in the fall collections. About 150 samples of *Lycopodium* sporophytes were collected at different places and in different seasons. In this study emphasis was placed on the living tissue, because the fungus is best visible here, but at the same time material of different samples was preserved in formal-alcohol and imbedded in paraffin and is available for future study.

DISTRIBUTION IN THE PLANT

Fungi were found to be distributed extensively on the surface and throughout the *Lycopodium* sporophyte. They were found in the roots, except close to the growing tip. In the rhizome they are particularly abundant and again extend near to, but do not invade the active growing point. Upright fruiting branches of such forms as *L. complanatum* var. *flabelliforme* have the fungus in the slender stalks bearing the cones.

In the Tissues

Cortex. The endophyte is found extensively developed in the cortical region of the rhizome. Strands of the hyphae cross the cortical cells and can be found penetrating the walls between adjacent cells. Intracellular loops and coils are frequent, especially in the older portions of the rhizome (Figs. 5 F, and 6 C, F, G, H). Some spherical fungus bodies are found occasionally and often the tiny motile cells are numerous (Fig. 6 D). The fungus is likely to be found in the cortical region containing starch. The cells with most abundant fungus usually have little or no starch, while nearby non-infected cells may be filled with it. *L. annotinum* rhizomes, in the late fall, unlike the other species studied, seemed to have their food reserves almost entirely in the form of oil as tested by Sudan III and I-KI.

The hyphae are usually non-septate, although on the surface of the rhizome, what appears to be the same type of fungus may have cross-

walls. Cross-walls are more apt to occur in regions containing fruiting bodies.

Xylem. In the spring samples, the fungus is especially easy to locate in the xylem cells of the central cylinder of the rhizome, and may be found within a short distance of the apical growing point. In the xylem the tiny motile cells are especially abundant at times, in the late summer and fall even completely filling the cells as seen in cross section (Fig. 6 D). Fungi are also present in the phloem but they are more difficult to observe in these small cells.

Toluidine Blue

When toluidine blue 0.1 per cent is added to the living material mounted in water on a slide, the *Lycopodium* cell walls stain blue to purple depending on the tissue involved. Xylem cells stain clear blue, as do living cortical cells. The non-living cortex cells stain purplish. Phloem does not stain at all. The fungus, unless injured or dead, does not stain with toluidine blue, which combined with the small size of the phloem cells makes it difficult to trace the fungus in that tissue. With iodine-potassium iodide solution, the fungus stains yellowish, indicating that the contents are of a protein nature. Small globules of fatty substance are present in the fungus, and stain red with Sudan III.

ISOLATION OF FUNGUS FROM SPOROPHYTES

Isolations have been made from rhizomes of *L. clavatum*, *L. obscurum*, and *L. complanatum* var. *flabelliforme*. Sections of rhizome in which living fungus is present, after being washed repeatedly in sterile distilled water, were placed in either distilled water or 2 per cent maltose solution. In a few days a fringe of mycelial hyphae usually grows out from the cut surface of the rhizome. If not sterile the fungus can usually be freed from bacteria and purified by sub-culturing. Fungi have also been isolated from the surface of *L. complanatum* var. *flabelliforme*, *L. lucidulum*, and *L. obscurum*, and from soil at stations of *L. obscurum* at Amherst and Smith Ferry Woods, Massachusetts.

A soil test was made Aug. 31, 1934, on the sample accompanying the *L. obscurum* gametophytes received from Junius, New York. Dilutions of 1 to 100 and 1 to 10,000 gave an average of 205,000 microörganisms per gram of soil. Both bacteria and fungi were present. Among the fungi recognized were *Mucor*, *Aspergillus*, *Fusarium*, and *Penicillium* (25). There also appeared in some of the cultures, the typical gray-green fungus obtained from *L. obscurum* gametophyte, with many conidia and some spherical bodies, and some small motile cells. The number of microörganisms on *Lycopodium* and in the forest soil where it grows, is almost legion. Besides the genera mentioned, various sooty molds are common. Flagellates, amoebae, nema-

todes, mites, etc., are often found. Seymour (26) lists a number of fungi reported on *Lycopodium*.

Isolations made from any one species at different times of the year seem to be a single form. On the surface of the *Lycopodium*, conidial spores of the gray-green type which seem to be associated with the endophyte are usually present.

Not enough work has been done at the present time to determine whether the fungi present in the sporophytes of the different species studied are identical, but at least it can be said, that the forms are very similar within the sporophytes as well as in cultures.

SUMMARY

1. A fungus was isolated from gametophytes of *L. obscurum* L. This fungus grows readily in pure culture on various media, and produces numerous gray-green conidia on aerial branches. Spherical bodies are also produced when enough moisture is present. Attempts are being made to identify the fungus.

2. Endophytic fungi have been found in about 150 samples of *Lycopodium* sporophytes including *L. annotinum* L., *L. clavatum* L., *L. complanatum* var. *flabelliforme* Fernald, *L. lucidulum* Michx., *L. obscurum* L., and *L. tristachyum* Pursh. These were collected from New York, Massachusetts, and Connecticut stations over a period of two years. The fungus is well distributed through the tissues, is found in the roots, and generally throughout the plant. It is particularly abundant in the rhizomes except in the growing points of the apical regions. *Lycopodium* sporophytes are thus added to the list of plants harboring an endophytic fungus.

3. Fungi were isolated from sporophytes of several species and when grown in culture media were found to be similar. It has not yet been determined whether they are identical. The general appearance is similar to that of the fungus isolated from the gametophyte of *L. obscurum* L. This study is still in progress.

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DEVELOPMENT OF THE FLORAL AXIS AND NEW BUD IN IMPORTED EASTER LILIES

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The relation to the life history of the origin and development of the flower axis was studied in two varieties of lilies commonly forced in the United States as Easter lilies, *Lilium longiflorum* Thunb. var. *eximium* Nichols (*L. eximium* Courtois), and var. *giganteum* Hort. In the trade, the variety *eximium*, which is the Bermuda lily, is known as *L. Harrisii* Carr. The variety *giganteum* is imported from Japan. Although a number of ornamental monocotyledons have been the subject of study, sometimes with practical application in forcing, these lilies have not previously received attention.

MATERIAL AND METHODS

Lilium longiflorum is classified under the sub-division Eulirion, characterized by Bailey as having almost horizontal trumpet-shaped flowers with a usually long tube made up of the perianth parts which are spreading only above the middle.

In practice the Bermuda variety is planted in the field in the fall, comes to bloom chiefly in March and April, and the bulbs are dug in August. The field-grown bulbs are dried and shipped, arriving in this country in August or September. They are kept in cool storage, that is, conditions comparable to a cool greenhouse or cellar, about 10° to 13° C., never lower than 5° or 6° C. The *giganteum* bulbs used were grown in northern Japan, where blooming occurs in late July and early August. They are dug at the end of August, and arrive in this country in October or November. They are kept in cold storage, usually at 0° C. or up to 3° C., prior to planting.

Ordinary cultural practice for growing lilies under glass indicates a temperature of 10° to 13° C. for a few weeks before transferring to a warmer house. This practice was followed except in the August planting of the variety *eximium*, which was placed directly in a warm house.

Lots of each variety of lily were available for three successive seasons.[1] Bulbs of the *giganteum* variety were received in December; in different years comparative study was made of material in cold storage (3° C.) and of bulbs potted at intervals from December 5 to April 16. Bulbs of the Bermuda lily were received in late August, September, or early October, and the stage of development was studied on the date of receipt. Comparison was made of bulbs planted in late August and early October in different years and of bulbs stored in a cool greenhouse from September 12 to No-

[1] Through the courtesy of Mr. Keith O'Leary.

vember 5. They were not carried longer in storage since these bulbs have been found to become dormant if held later than November.

Bulbs of both varieties bear scales from more than one season. There is a general relation between the size of the bulb and the number of scales. The following counts are exclusive of the few innermost organs left about the bud in pickling it. Bulbs of the 1932 lot of the variety *eximium* had up to 100 scales and were listed as 11 to 13 inches circumference; the 1933 and 1934 bulbs, both listed as 9 to 11 inches, had about 80 to 90 scales and 50 to 60 scales respectively. For all three seasons, the *giganteum* bulbs were 7 to 9 inches; they varied in scale count as follows: 50 to 70, 55 to 75, and 45 to 65.

Examinations were made at convenient close intervals. In controls, two to five bulbs were used at one time and in subsequent examinations, one, two, or three were dissected at a time. They were in some cases dissected prior to pickling so that the condition of the bud could be seen with low magnification. Because of possible damage to the tender watery tissues and loss of relationship of parts, later technique avoided such extreme dissection, and the buds with a number of young leaf parts were slabbed and pickled. The killing fluid most frequently used was a formalin-acetic-alcohol mixture, with occasional use of the Allen-Wilson modification of Bouin's fluid. Material was imbedded in paraffin, and sections were cut in longitudinal and transverse planes.

RESULTS

It was found that in both varieties there is some development during storage at 3° C. for var. *giganteum*, and 10° to 13° C. for var. *eximium*. Earlier stages were available in variety *eximium*, later stages in variety *giganteum*, and intermediate in both varieties.

Variety eximium. In late August the apex of the stem appeared almost plane in the Bermuda lily bulbs, and the organs which were in the process of development were leaf structures (Fig. 1 A and B). Of these the youngest primordia did not cover the stem apex. The interval between this and the next observation was adequate to change the form to a rounded apex (Fig. 1 C and D). In some bulbs, leaves were still being formed, in others the phase of leaf elongation was evident so that in sections the innermost leaves exceeded the height of the stem apex, overlapping it. Meanwhile there was very slow elongation of the axis proper. Gradually growth of the stem tissues resulted in a broadening of the apex, the first evidence of change from a vegetative to a reproductive condition. This broad tip was found in buds in which elongation of the stem was not readily discernible and represents a pre-differentiation stage of the inflorescence. This appears to be as far as development goes in storage in variety *eximium* (Fig. 1 E). But when bulbs were planted, these stages followed each other more rapidly, and

there was more evident elongation of the axis and leaves. The apex of the stem was elevated by the growth of the internodes and it soon passed beyond the stage seen in stored material (Fig. 1 F to L). The floral parts of the first flower, terminal in position, began to differentiate. The second flower rudiment developed, and soon others in succession.

Variety giganteum. In the variety *giganteum*, the early stages were not seen. By the time bulbs came to observation in early December, the apex had presumably passed through the stage of a flat apex seen in the Bermuda lily at the early dates, and had already taken on the rounded form (Fig. 2 M). The extent of elongation of the axis was variable (Fig. 2 N) but there was a tendency toward greater elongation than in storage stages of variety *eximium* so that in some samples the axis was over a centimeter long by mid-April (Fig. 2 O). In planted bulbs the elongation of the axis was slow at first (Fig. 2 P) but progressed much more rapidly a few weeks after planting (Fig. 2 R). The pre-differentiation stage was succeeded by that of differentiation of the first flower, with formation of outer perianth parts (Fig. 2 P, R, S). This occurred before the initiation of the second primordium as a prominence in the axil of a bract below the first flower (Fig. 2 T and V). There was continued growth and differentiation of the floral parts in the successive flower primordia, so that several stages of floral development were seen in the different flowers in a single head (Fig. 2 V). The growing period preceding the appearance of any definite stage, for example the second primordium, was shortened with longer storage intervals before planting.

The order of development in the flower after the bracteole is formed, is an outer perianth set, followed by alternating members of the inner perianth cycle, and then two cycles of stamens opposite the individual perianth parts. There is somewhat slower development of the three protuberances representing the carpels which grow to form the pistil (Fig. 2 U). These are opposite the outer perianth parts. Growth of partitions that form opposite the inner perianth members produce the three-chambered ovary.

Relation of stages to time. Even when bulbs are selected for size a lot is is apt to show some difference in degree of development at any one time. There was evidence of this lack of uniformity both from tests where a number of bulbs were examined at one time, and in the appearance of a single stage at different times. But as a result of frequent sampling in periods deemed to be critical from experience in the first lots it is possible to construct a sort of time schedule for these varieties.

Variety eximium. In bulbs of variety *eximium* in cool storage the vegetative condition was seen October 2 and 17 in two samples. On October 22, the apex was wider, and on October 31, slight elongation of the axis was visible in one bulb but the rudiments differentiated were still leaf structures (Fig. 1 E). Observation continued to November 5, with no further develop-

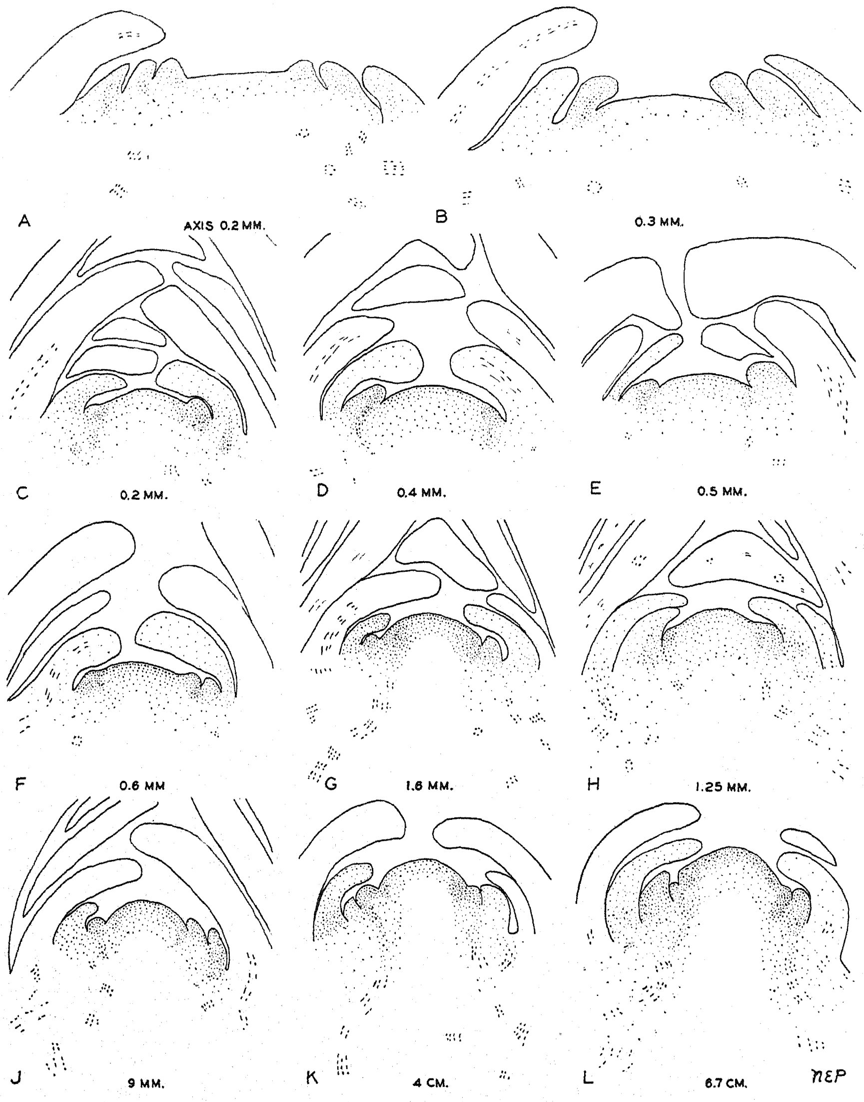

FIGURE 1. Longitudinal sections of terminal buds of *Lilium longiflorum* var. *eximium*. A to E. Stages in storage. A and B. August 24. C. Sept. 12. D. Sept. 24. E. Oct. 31. F to J. Bulbs potted Oct. 4. F. Pre-differentiation stage, 13 days. G and H. Axis elongating, 18 days and 27 days. J. Perianth parts developing, 53 days. K and L. Bulbs potted Oct. 16; further development of first flower primordium, 66 and 87 days. Magnification in Figures A to X is 28.8; in Y is 1/2.

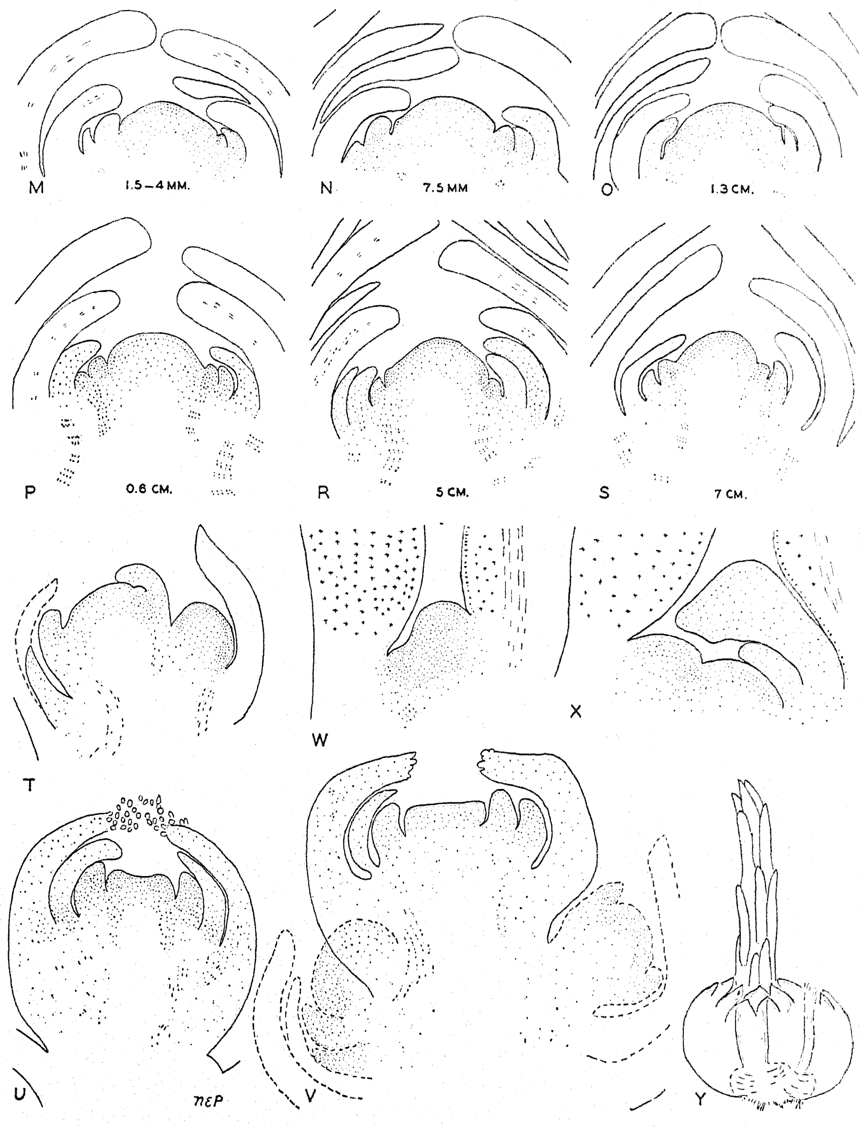

FIGURE 2. *L. longiflorum* var. *giganteum*. M to V. Terminal bud. M, N, O. Storage, pre-differentiation stage, Dec. 4, Jan. 31, Apr. 16. P to S. Differentiation of first flower. P, R. Dec. 5 series, 15 and 36 days. S, T, U. Feb. 15 series, slow bulbs. S. 46 days, first flower. T. 53 days, two primordia, U. 46 days, oldest of three primordia, showing all organs. V to Y. Apr. 16 series. V. Relation of primordia, 31 days. W. Young bud in axil of scale at base of floral axis, 19 days. X. Later stage of bud, same plant as V. Y. Diagram of relations of present and old floral axes, bulb axis and scales, same plant.

ment of the axis or elongation of the apex in storage material. Bulbs from the same lot that had been planted October 4 showed extension of the leaves and slight elongation of the axis on October 17 (Fig. 1 F). In five more days, the apex appeared full rounded (Fig. 1 G), due to upward growth. Floral differentiation had begun. In 32 and 46 days, there was further advance, with all outer perianth parts developing. Marked elongation of the stem was observed November 19 and 26 (Fig. 1 J), intervals of 46 and 53 days after planting. The first flower primordium was developing, but there was no evidence of a second as yet. When this series was discontinued at 77 days, there had been no further progress.

Bulbs potted on October 16 in another year showed elongation resulting in an axis about 4 cm. long in 66 days (Fig. 1 K) and 6.7 cm. in 87 days (Fig. 1 L). In these the floral parts of the first primordium showed greater development. The second primordium was seen 99 days after planting, and in 137 days there were visible three flower buds in the oldest of which all four organs were differentiated.

Bulbs planted earlier in the season (August 24) were vegetative when planted (Fig. 1 A and B) and when first examined in 54 days already showed a broad apex, development of floral parts, and an axis elongated to 3.5 to 4.5 cm. Obviously the first stages of differentiation were missed here. Examination on three later dates showed increased size and differentiation of the first flower. Four primordia were seen in plants examined 107 and 115 days after planting, and blooming occurred in 145 days. Since these bulbs were started at a higher temperature, no exact comparison can be made with the set planted seven weeks later.

Variety giganteum. At the time of earliest examination (December 4) the variety *giganteum* bulbs were already in the stage of a broad apex, the pre-differentiation stage (Fig. 2 M). It is therefore impossible to state when the change from strictly vegetative to incipient flowering condition occurred. There had also been elongation in some axes, with measurements in four bulbs 0.15, 0.4, 0.6, and even 1.0 cm. for total length. Stored bulbs examined at close intervals until the middle of February had the following lengths: 0.4 cm., December 20; 0.6 cm., January 17; 0.75 cm., January 31 (Fig. 2 N); 0.37 cm., February 11; and 0.7 to 0.8 cm., February 18. Bulbs in storage until April 16 (Fig. 2 O) showed increased stem length, in some samples as much as 1.3 to 2 cm. In the longest stem, differentiation of the first floral parts was apparently beginning.

Bulbs of the same lot planted on December 5, or held in cold storage and planted January 10, showed in both series a relatively slow elongation of the stem (Fig. 2 P) during the first two or three weeks after planting, and then rapid growth, 5 cm. long a month after planting (Fig. 2 R). Along with marked elongation there was differentiation of the parts of the flower primordium. In the December planting, the differentiation of the stamen

sets had not taken place at the last observation in 36 days, nor was a growing region for the second primordium distinguishable. In the January planting, four primordia were visible in 31 days. In a planting on December 5 of another year there were easily visible flower buds in many plants in 86 days, in slower plants, three flower primordia with the oldest showing carpel differentiation. A planting on February 15 showed wide variation, but all except the slower plants had several primordia, often with carpel differentiation in the oldest flower of each plant, in 46 days (Fig. 2 U). Slower plants selected from this lot are represented in Figure 2 S and T. Bulbs planted April 10 showed two primordia in 24 days while still others of April 16 showed the second primordium beginning in 12 days and three primordia in 17 days. It is apparent that the later the planting, the shorter the time thereafter for the production of the second or any subsequent primordium. Differentiation in the first flower is also associated with this although it is more difficult to determine the first stages. Formation of the depression marking pistil development (Fig. 2 U) is a more definite stage and the shortest interval in which it developed in the first flower was found to be in the latest planting. The interval to blooming was also shortened. The shorter time required after planting for any specific stage is undoubtedly associated with progress made in the seemingly dormant bulbs during storage. Examination of the last bulbs planted made it clear that there was morphological development evidenced by increased size of apex and length of axis over any previous storage stages. The physiological changes associated with this were not studied at this time.

Formation of the new bud. The very early stages which lead eventually to the production of the next year's growth were observed in variety *giganteum*. The bud which is to function in making the following year's floral axis arises in the axil of the last of the scale leaves on the bulb axis; occasionally there are two buds in two leaf axils, with splitting of the bulb. In the cases examined, the bud was diametrically opposite or at one side with relation to the position of the old floral axis.

The earliest stage seen in variety *giganteum* was a prominence of meristematic tissue seen in dissections of month-old plants of a December planting and 17-day old plants of an April planting (Fig. 2 W). The shoots for this year's flowering were just emerging from the soil or a few centimeters above the soil; this represents a stem length of 3.2 to 6.5 cm. from the bulb axis. In the most advanced plants, two or three flower primordia were seen at the apex. In some cases roots had not yet appeared at the surface, although some between the scales were a centimeter long. In the plant diagrammed in Figure 2 Y, the first leaf member had been formed on the side of the bud toward the functional axis and two other members were visible in the section (Fig. 2 X). This plant, observed 29 days after planting in April, had a shoot 7.7 cm. in length from the bulb axis, with 2.4 cm.

above the soil level. In the oldest flower primordium (Fig. 2 V) outer and inner perianth and stamen cycles were differentiated and the carpel set was in process of differentiation. Of the roots now visible, the longest was 4 cm. in length. Other leaf or scale primordia followed. Young bulbs examined about nine weeks after February planting were from 1 to 1.4 cm. long, and usually less than 1 cm. in the widest diameter. A cross section of one of two buds occurring at the base of the floral axis, showed a dozen leaf scales. Shoots above ground measured about 11 to 15 cm. in height and there was strong development of the roots arising from the axis above the level of the old bulb. In the oldest flower primordium, the sepals had become large enough to cover completely the other floral parts. A bulb in approximately the same stage was seen in a ten-week-old plant started December 4. This bulb was appressed against the erect stem and consisted of 14 scale leaves. In section it showed active formation of new leaf primordia.

The new bud originates about as early in the variety *eximium*. A very small one observed in a three-week-old plant of an October planting, occurred in the axil of a thick scale. All the scales contain much starch. Here the vertical axis was still short, with the rounded apex surrounded by the crowded thinner and somewhat elongated leaves. No attempt was made to follow the development, which presumably is like that of variety *giganteum*.

DISCUSSION

These two varieties of *Lilium longiflorum* go through the stages of leaf formation, flower formation, extension and rest period reported for other liliaceous plants, but with different time relations. In *Narcissus* the growing region initiated in May (5) or flowering time in spring (7) develops scale and leaf structures until the same time in the following year, when there is the beginning of flower formation. Differentiation is completed in several months (late July or August). In *Hyacinthus orientalis* (1) the new vegetative growth starts at the end of July or beginning of August, in the Darwin tulip (8) in late July, and goes on for twelve months, when the change to the flowering condition occurs. There is complete differentiation of the flower parts (tetrad stage in stamens) by October; during the winter there is no further development of these parts, although the bulb axis and floral axis grow very slowly. Blooming occurs the following spring, approximately two years after initiation of the growing region leading to that particular inflorescence. *Convallaria majalis*, lily of the valley, (14) differs in that the leaf cluster and flower cluster appearing at one time belong to two different axes, instead of representing development of a single one. But two years elapse between the origin and the final stage of the scale leaves. The formation of the flower cluster begins in April of one year for functioning the next spring, approximately the same length of time for growth and differentiation as in those first mentioned.

In the lily varieties here studied the new vegetative region appears soon after planting and develops a small bulb within the scale leaves of the present season's growth while this year's flowers are developing. After spring flowering, the vegetative growth of the new bud continues in variety *eximium* until the following fall when the change to floral condition is initiated; this change was not seen in variety *giganteum*, but must begin earlier than December. The floral axis grows slowly in storage, more rapidly as soon as the bulb is planted, and by the time it is 3 to 6 cm. in length the new vegetative structure is seen. Thus there is approximately a year's development between initiation of the growing region and the production of flower primordia. The interval necessary for full development of these primordia, that is, the blooming stage, is several additional months. It is clear that during a part of the year two growing axes are present in the bulb, but from the time of death of the current flower stalk until shortly after planting only one is present.

Lilium candidum (6) and *L. Martagon* (12), summer-blooming species, both show in the fall the young bud which is to form flowers the second summer hence. According to Rimbach, next year's axis in *L. Martagon* is 2 cm. long as compared with a 2 to 3 mm. bud for the succeeding year. Irmisch reported that next year's axis bore flower primordia at this time in *L. candidum*. Here the resting period occurs after the formation of both new bud and flower primordia, so that a longer time elapses between initiation of the growing point and blooming.

The interval from flower initiation to complete differentiation of the flower parts, approximately two or three months here, may be reduced even more in such forms as *Iris* where one variety (2) goes through these stages in five weeks (from March 1 to April 7) and blooms by the end of May. No account is given as to the exact time of first initiation of this growing region; leaf production however went on during the preceding summer and was completed during the winter months immediately previous to differentiation of floral parts.

Comparison may also be made with *Gladiolus* which remains in the vegetative phase throughout storage (10, 13). When corms are forced for winter growth, the last leaves may be in the process of formation at the time of planting; within 40 days in one variety, elongation of the stem apex had begun and within 47 days the first flower primordia were seen (10). Spring planting in the field brought about rapid change, with elongation of the floral axis commencing about two weeks after planting, and rapid formation of the flower spike; carpel formation in the lowermost flower began about five or six weeks after planting, depending upon the variety (11). In both *Iris* and *Gladiolus*, as in lily, the first growth and differentiation may occur when low temperatures prevail.

In considering the inflorescence of *Lilium longiflorum* the relation of the flowers is found to be as described in *L. Martagon* (6). The first flower

is terminal and succeeding ones are produced in the axil of the bracteole on the stalk of the next older flower. Substantially the same relations were described in *L. candidum* by Payer (9) who also figured in detail the order of development of the flower parts, beginning with the formation of the bract and of the bracteole to one side of it. The first perianth part arises as a swelling on the side diametrically opposite the bracteole, and a second and third member of this cycle are produced, of which the last occurs on the same side as the bract (anterior). The inner perianth parts also appear in succession, alternating with the outer members, with the first inner between the outer first and second, the next between the second and third, and the last between the first and third outer. The stamens occur in two cycles of three each, of which the outer members develop opposite the outer perianth, the inner opposite the inner perianth members. Later three protuberances appearing opposite the outer perianth divisions become joined and form the elongated pistil. The partitions of the ovary are produced by growth of three folds of tissue from the ovary wall at points opposite the inner perianth parts. Ovules appear in two series from base to apex in each of the three cells. Eichler (3, vol. 1, p. 153–155) diagrammed the arrangement of floral parts in *L. bulbiferum*, with the bracteole either diagonally backward or lateral to the bract. Hofmeister (4, pp. 470, 506) showed the arrangement of the floral parts as oblique with relation to the bract in *L. candidum* due to the position of the bracteole (diagonally backward from bract position). No attempt was made to follow in as great detail the development of floral parts in the two varieties of *L. longiflorum* but no conflicting evidence resulted from study of preparations available.

SUMMARY

In *Lilium longiflorum* Thunb. var. *eximium* Nichols the vegetative stage seen in August persists in cool storage (10° to 13° C.) until about the middle of October. A broadening of the apex and slow elongation of the stem indicate the pre-differentiation stage of the floral axis. In bulbs held in cool storage until November 26, this development is very gradual.

The rate of growth increased in planted bulbs, even at similar temperatures, but did not become marked until several weeks after planting.

In *L. longiflorum* Thunb. var. *giganteum* Hort. the apex was already broad at the time of first observation in early December. In storage thereafter at 3° C. there was slow elongation of the axis. Usually there was no differentiation of floral parts in storage, but rarely with greater elongation, differentiation of the first flower primordium apparently began with storage until April 16.

Planting resulted in an increased rate of activity both in elongation of axis and differentiation of first flower parts, within two or three weeks after potting; growth in length became marked within three or four weeks.

The interval necessary after planting for completion of the first primordium is shorter with longer storage, due to progressive although slow changes during storage.

The order of development of floral members is acropetal; in both varieties, the carpel set is slow in appearing and the second and third primordia are visible before it is well differentiated in the first flower.

The microscopic bud which is to produce the bulb functioning in the succeeding season is visible at the base of the present year's floral axis when this is 3 to 6 cm. long. This bud occurs in the axil of the last scale leaf on the old bulb axis, usually diametrically opposite or to the side, with relation to the position of the previous year's floral axis. Occasionally two buds occur.

The first scale of the new bulb is produced on the side toward the present floral axis.

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STORAGE OF VEGETABLE SEEDS

Lela V. Barton

The storage of vegetable seeds so that vitality may be retained over a period of years is a vital problem to both seedsmen and growers. The consideration of successful storage conditions must include temperature, moisture content, and oxygen supply. The effectiveness of the method depends on the interrelation of these factors rather than on a single factor.

Since the literature on seed storage is very extensive, the discussion here will be confined to articles which deal with storage of crop and vegetable seeds with special emphasis on the latter.

One of the earliest reports on this subject was that of Carruthers (3) who stored 43 kinds of farm seeds in paper bags in cabinet drawers. His tests showed that the seeds varied in the rapidity of the decline in germination. Carrot seeds lost their vitality at a uniform rate and gave no germination the tenth year.

Lafferty (10) found a small but gradual decrease in the percentage of germination of some grain seeds stored in paper bags in the laboratory, during the early years of the trial. This was followed by more rapid decreases until the seeds were dead. The experiment was continued over a period of 15 years. Robertson and Lute (13) stored grains in 100-pound bags in an unheated room and obtained results similar to those of Lafferty.

Whymper and Bradley (21, 22, 23) in a series of studies on the vitality of wheat seeds found that desiccation over $CaCl_2$ was conducive to the retention of vitality.

Sonavne's (17) experiment with 14 species of crop seeds showed excellent germination after five years of storage, with the exception of peanut. He placed seeds in sealed glass bottles with a naphthalene ball in each. After 12 years [Sonavne (18)], germination was not affected by the naphthalene.

Oathout (11) stressed the importance of moisture content in his work on soybeans. If the normal water content (10 to 14 per cent) was maintained, the vitality remained unimpaired for more than two years regardless of the conditions under which they were stored. If, however, the water content appreciably exceeded 14 per cent, good ventilation or low temperature, or both, became necessary. Spencer (19) also found temperature and humidity important factors in the storage of soybeans and peanuts in the tropics. Although his experiment was continued for only ten months, the beneficial effects of cool storage were striking in the case of the soybeans and significant in the case of the peanuts. On the other hand, Beattie (1) found that low temperature did not prolong the life of peanut seeds when they were stored in muslin bags in large galvanized cans.

Joseph (9) working with parsnip seeds cited the beneficial effect of low temperature and desiccation on keeping quality.

Rose (14) in his report of a study of delayed germination in economic seeds remarked that two varieties of lettuce seed improved in viability as they grew older, at least up to the end of the fourth year. This improvement, he said, was due to the increased permeability of the inner seed coat.

In his progress report on various kinds of seeds harvested in 1924 and stored through 1932, Pritchard (12) gave carrot, cauliflower, lettuce, and onion as seeds which lost their germinative ability to a great extent while tomatoes were among those retaining their vitality very well.

The present paper is a preliminary report on the effects of different storage conditions on the retention of vitality of some vegetable seeds. A later report will furnish further data obtained from these same seeds, a considerable quantity of which still remain in storage.

MATERIAL AND METHODS

Seeds of carrot (*Daucus carota* L. var. *sativa* DC.), cauliflower (*Brassica oleracea* L. var. *botrytis* L.), eggplant (*Solanum melongena* L.), lettuce (*Lactuca sativa* L.), onion (*Allium cepa* L.), pepper (*Capsicum frutescens* L. [*C. annuum* L.]), and tomato (*Lycopersicon esculentum* Mill.) were obtained from the Kilgore Seed Company, Plant City, Florida, in May 1932. These seeds had been stored at room temperature up to the time of shipment. As soon as they were received, moisture determinations were made and germination and seedling production tests were conducted.

One lot of seeds was stored at the Institute at room temperature and at $-5°$ C. Another lot was sent back to Florida to be stored there under warehouse conditions. Samples of each kind of seeds with the original moisture content were stored opened and sealed. In other samples part of the moisture was removed, either by drying in a desiccator over calcium oxide until about one-third of the moisture was removed, or by mixing directly with the amount of calcium oxide required to remove one-third and one-half of the moisture present in the seeds. All of the dried lots were sealed and stored at the two temperatures mentioned above.

Sealed storage was in tin cans with tight-fitting lids, sealed with Dekhotinsky cement. Care was taken not to heat the seeds in sealing. Since the supply of cauliflower seeds was limited, storage was made without drying and at room temperature only in open and sealed containers.

Tests for vitality were conducted both in electrically-controlled ovens and in the greenhouse. The oven tests were made on moist filter paper in petri dishes while the greenhouse tests were made in soil composed of peat, sand, and sod soil. In the former case the appearance of the hypocotyl was taken as the criterion of germination, while in the greenhouse actual seedling production, or the emergence of the shoot above ground, was re-

corded. This distinction is necessary especially in seeds that have been stored for some time as old seeds often possess enough energy to send out a short root but cannot produce seedlings in a soil planting. Duplicates of 100 seeds each were used for each germination test.

Various oven temperatures were used according to the specific temperature requirements of the different seeds, but all soil plantings were made in the same greenhouse in May of each year. The greenhouse was kept at 70° F., except of course for the warmer days when it was impossible to control the temperature.

GERMINATION OF "FRESH" SEEDS

All of the seeds were tested for germinative ability when they were first received in this laboratory. In most cases the oven temperature shown by previous tests to be best was used. However, the optimum temperature for carrot seed was not known. Hence an experiment using different temperatures was made. These seeds were found to germinate over a wide range of temperatures. From 60 to 71 per cent germination was obtained at constant temperatures of 15°, 20°, 25°, or 30° C. or at daily alternating temperatures of 15° to 30° C. or 20° to 30° C. The daily alternation of 15° to 30° C. was selected as the temperature to be used in subsequent tests.

Since previous workers [Borthwick (2), Cole (4), Franck and Wieringa (8), Shuck (15), and Towers (20)] had reported a beneficial effect of a pretreatment at low temperatures on the germination of lettuce seed, this method was tried. Cultures were placed on moist filter paper at 1,° 5°, or 10° C. and transferred to 15° to 30° C. daily alternation after four days. Germination obtained after such treatment was from 89 to 96 per cent. Subsequent transfers after longer times at low temperatures were not possible since the germination was quite heavy at 10° C. after five days and at 1° C. and 5° C. after nine and seven days. If the seeds were placed at 15° to 30° C. daily alternation without any previous low temperature treatment a germination of 98 per cent was obtained in four days. The effect of light at room temperature was also tried [Flint (5, 6, 7), Shuck (15, 16)] but the germination was very poor, only 8 per cent. This was probably due to the unfavorable room temperature, because a pre-treatment in light followed by a favorable temperature was effective in later tests. All of these results with lettuce seed indicated that the lettuce seed received here was of high germination capacity and needed no special treatment. Other special germination tests were made after two and three years of storage and will be reported below.

The results of the oven tests given in Table I are for the following temperatures for the various seeds: carrot, 15° to 30° C. daily alternation; cauliflower, 25° C. constant; eggplant, 20° to 30° C. daily alternation;

TABLE I

GERMINATION PERCENTAGES AFTER STORAGE UNDER VARIOUS CONDITIONS

Seed	Storage condition	Fresh	Stored at room temp.			Stored at $-5°$ C.		
			1 yr.	2 yrs.	3 yrs.	1 yr.	2 yrs.	3 yrs.
Carrot 10.7%*	Air-dry open	67	66	62	63	68	69	73
	Air-dry sealed		60	60	25	67	71	75
	Dried over CaO to remove about 1/3 of moisture sealed		62	65	60	67	71	66
	Mixed with CaO to remove 1/3 of moisture sealed		59	65	65	65	67	76
	Mixed with CaO to remove 1/2 of moisture sealed		63	72	64	69	71	71
Eggplant 10.4%*	Air-dry open	86	81	90	82	82	85	84
	Air-dry sealed		86	74	83	85	87	85
	Dried over CaO to remove about 1/3 of moisture sealed		87	90	88	86	90	86
	Mixed with CaO to remove 1/3 of moisture sealed		87	86	77	79	82	86
	Mixed with CaO to remove 1/2 of moisture sealed		93	88	89	82	83	82
Lettuce 8.2%*	Air-dry open	98	95	89	76	94	94	94
	Air-dry sealed		92	91	85	95	96	93
	Dried over CaO to remove about 1/3 of moisture sealed		92	97	88	91	94	96
	Mixed with CaO to remove 1/3 of moisture sealed		94	93	91	94	93	95
	Mixed with CaO to remove 1/2 of moisture sealed		94	89	88	91	95	95
Onion 12.5%*	Air-dry open	98	62	69	33	94	96	94
	Air-dry sealed		82	1	1	96	94	97
	Dried over CaO to remove about 1/3 of moisture sealed		90	96	91	94	94	97
	Mixed with CaO to remove 1/3 of moisture sealed		96	93	87	95	93	94
	Mixed with CaO to remove 1/2 of moisture sealed		96	95	99	97	96	97
Pepper 10.4%*	Air-dry open	73	67	64	45	80	86	73
	Air-dry sealed		22	0	2	75	86	76
	Dried over CaO to remove about 1/3 of moisture sealed		74	74	65	70	81	81
	Mixed with CaO to remove 1/3 of moisture sealed		74	74	68	76	73	77
	Mixed with CaO to remove 1/2 of moisture sealed		76	74	66	43	73	74
Tomato 10%*	Air-dry open	93	94	89	89	92	90	91
	Air-dry sealed		91	84	75	93	89	91
	Dried over CaO to remove about 1/3 of moisture sealed		87	87	87	92	87	90
	Mixed with CaO to remove 1/3 of moisture sealed		91	92	93	90	90	85
	Mixed with CaO to remove 1/2 of moisture sealed		90	89	91	90	91	85
Cauliflower	Air-dry sealed	84	61	12	0	—	—	—
	Dried over CaO to remove about 1/3 of moisture sealed		77	83	74	—	—	—

* Percentage of moisture (on basis of dry weight) in seeds at the beginning of the experiment.

TABLE II

SEEDLING PRODUCTION IN SOIL IN GREENHOUSE AFTER STORAGE UNDER VARIOUS CONDITIONS

Seed	Storage condition	Fresh	Stored at room temp.			Stored at −5°C.		
			1 yr.	2 yrs.	3 yrs.	1 yr.	2 yrs.	3 yrs.
Carrot 10.7%*	Air-dry open	54	46	43	44	42	59	49
	Air-dry sealed		44	29	2	46	67	58
	Dried over CaO to remove about 1/3 of moisture sealed		48	43	54	32	54	48
	Mixed with CaO to remove 1/3 of moisture sealed		48	35	46	39	51	54
	Mixed with CaO to remove 1/2 of moisture sealed		49	44	44	30	57	55
Eggplant 10.4%*	Air-dry open	76	76	63	68	72	75	69
	Air-dry sealed		67	73	70	80	74	77
	Dried over CaO to remove about 1/3 of moisture sealed		77	79	64	78	76	75
	Mixed with CaO to remove 1/3 of moisture sealed		75	79	71	76	76	76
	Mixed with CaO to remove 1/2 of moisture sealed		77	83	63	69	84	78
Lettuce 8.2%*	Air-dry open	13	28	72	23	21	29	73
	Air-dry sealed		14	41	14	17	42	79
	Dried over CaO to remove about 1/3 of moisture sealed		10	29	69	10	22	76
	Mixed with CaO to remove 1/3 of moisture sealed		11	53	68	17	13	82
	Mixed with CaO to remove 1/2 of moisture sealed		8	30	68	18	26	78
Onion 12.5%*	Air-dry open	92	79	50	13	86	84	86
	Air-dry sealed		58	0	0	86	94	91
	Dried over CaO to remove about 1/3 of moisture sealed		83	80	82	88	91	86
	Mixed with CaO to remove 1/3 of moisture sealed		88	86	79	88	93	87
	Mixed with CaO to remove 1/2 of moisture sealed		88	90	87	92	91	89
Pepper 10.4%*	Air-dry open	63	58	42	17	34	61	61
	Air-dry sealed		10	0	0	35	79	61
	Dried over CaO to remove about 1/3 of moisture sealed		64	60	30	20	79	58
	Mixed with CaO to remove 1/3 of moisture sealed		66	64	36	42	35	58
	Mixed with CaO to remove 1/2 of moisture sealed		60	64	52	40	36	48
Tomato 10%*	Air-dry open	82	84	72	72	71	62	83
	Air-dry sealed		81	72	55	74	76	89
	Dried over CaO to remove about 1/3 of moisture sealed		87	76	80	81	69	81
	Mixed with CaO to remove 1/3 of moisture sealed		82	80	72	58	74	79
	Mixed with CaO to remove 1/2 of moisture sealed		77	76	76	50	71	85
Cauliflower	Air-dry sealed	74	36	4	1	—	—	—
	Dried over CaO to remove about 1/3 of moisture sealed		58	43	52	—	—	—

* Percentage of moisture (on basis of dry weight) in seeds at the beginning of the experiment.

lettuce, 15° to 30° C. daily alternation; onion, 25° C. constant; pepper, 25° C. constant; tomato, 25° C. constant.

It will be noted from Tables I and II that the germination obtained in the ovens was always superior to the seedling production in the greenhouse. In some cases, this difference was quite marked. Lettuce was most affected in this regard, giving 98 per cent germination in the oven and only 13 per cent seedling production in the greenhouse. In other forms, the seedling production compared very favorably with germination. This was especially notable in the case of the onion (92 and 98 per cent).

GERMINATION AFTER STORAGE

SEEDS WITH VITALITY UNIMPAIRED

Eggplant. These seeds retained their germinating and seedling-producing power perfectly in all storage conditions for three years (Tables I and II).

Tomato. Tomato seeds kept well in all conditions, but after three years of storage both germination and seedling production had fallen off slightly in the undried, sealed condition at room temperature (Tables I and II). This decrease will no doubt become more marked with a longer period of storage.

SEEDS BENEFITED BY DRYING

Carrot. It will be seen from Tables I and II that carrot seeds from all storage conditions germinated as well after one year of storage as they did in the initial test. Hence, within the limits of this experiment, neither moisture content nor storage temperature had any effect on the vitality of these seeds in one year.

When they were tested after two years' storage, however, there was some indication that −5° C. was superior to room temperature for retention of vitality especially as measured by seedling production in the greenhouse. At this storage temperature the moisture content appeared to be of no significance. After three years of storage, however, drying was decidedly beneficial when the seeds were stored sealed at room temperature.

Lettuce. In spite of the fact that excellent germination was obtained from all storage conditions after one, two, and three years of storage, the seedling production was much lower, especially after one or two years (Table II). This was noticeable even though the seedling production after these storage periods either equalled or exceeded that obtained from the initial test. However, a soil test after two years of storage yielded many more seedlings than did a similar test of "fresh" seeds or seeds stored one year. Improved seedling production was still to be noted after three years of storage. This improvement was more marked for seeds stored at −5° C. than for those stored at room temperature. At this time, however, seeds

stored open or sealed air-dry showed a distinct lag in seedling-producing powers, as compared with more favorable storage conditions. Under favorable storage conditions the seedling production of lettuce seeds in the soil increased from 13 per cent for "fresh" seeds to as high as 82 per cent for three-year-old seeds, while the germination percentages were high from the first and remained practically the same throughout. Rose (14) noted improvement in percentage germination of lettuce seeds with age.

Since the germination percentages far exceeded those for seedling production, it was decided to give the seeds in room temperature storage pre-treatments before planting in soil after the two-year storage period. Pre-treatments given were as follows: 1. Exposure to light in water for four hours. 2. Injection with water for 15 minutes. 3. Treatment at 5° C. (on moist filter paper) for five days. 4. Storage in moist chamber for five days. One lot was also planted in soil and put in the ovens at a daily alternating temperature of 15° to 30° C. The results are shown in Table III. All of the pre-treatments produced much better stands of seedlings than when the seeds were planted directly. These tests were made in June instead of May, consequently a new control was planted. All storage methods used here increased the seedling production energy of lettuce seeds, which energy could be still further increased by special pre-treatment.

TABLE III

SEEDLING PRODUCTION FROM LETTUCE SEEDS STORED TWO YEARS AT ROOM TEMPERATURE

Storage condition	% seedling production after pre-treatment				
	Light 4 hrs.	Water injection 15 min.	5° C. for 5 days	Moist chamber 5 days	Check
Air-dry open	53	37	68	61	57
Air-dry sealed	27	49	65	26	7
Dried over CaO to remove about 1/3 of moisture sealed	49	55	86	47	16
Mixed with CaO to remove 1/3 of moisture sealed	71	73	78	60	20
Mixed with CaO to remove 1/2 of moisture sealed	69	63	71	35	7

The light and low temperature pre-treatments were given again after three years of storage to seeds from both room temperature and −5° C. storage. As would be expected from the natural increased seedling productive power at this time, the differences between pre-treated and untreated seeds were not so great (Table IV), but the low temperature treatment increased seedling production.

Onion. A reference to Tables I and II will show that onion seeds retained their vitality remarkably well in all conditions at −5° C., and in sealed storage at room temperature provided they had been dried before

storing. Seedling production from air-dry seeds sealed at room temperature dropped after one year of storage and both germination and seedling production were nil after two and three years (Tables I and II).

TABLE IV

SEEDLING PRODUCTION FROM LETTUCE SEEDS STORED UNDER VARIOUS CONDITIONS FOR THREE YEARS

Storage condition	% seedling production after pre-treatment		
	Light 4 hrs.	5° C. for 5 days	Check
Stored at room temperature			
Air-dry open	22	13	23
Air-dry sealed	31	23	14
Dried over CaO to remove about 1/3 of moisture sealed	68	79	69
Mixed with CaO to remove 1/3 of moisture sealed	72	89	68
Mixed with CaO to remove 1/2 of moisture sealed	67	84	68
Stored at −5° C.			
Air-dry open	84	88	73
Air-dry sealed	80	94	79
Dried over CaO to remove about 1/3 of moisture sealed	74	89	76
Mixed with CaO to remove 1/3 of moisture sealed	61	89	82
Mixed with CaO to remove 1 2 of moisture sealed	75	90	78

Open storage at room temperature was also inferior to sealed storage of dried seeds, but was decidedly superior to sealed storage of air-dry seeds. After three years of storage, however, there was a decided decline in germinative power of seeds stored in open containers.

Pepper. Results (Tables I and II) indicated a condition similar to that shown by onion. The seedling production in this case, however, did not always keep pace with the germination and became noticeably reduced after three years of storage.

Cauliflower. The small quantity of these seeds prevented extensive experiments but the results from sealed storage with initial water content as compared with those from sealed storage with previous drying over CaO showed the favorable effect of desiccation (Tables I and II). These seeds were stored at room temperature only. It appears then that the storage requirements of this form are similar to those for carrot, lettuce, onion, and pepper seeds.

SUMMARY

Some vegetable seeds were stored open and sealed at room temperature and −5° C. In some cases, seeds were dried over calcium oxide to remove about one-third of the initial water content or mixed with quantities of calcium oxide sufficient to remove one-third and one-half of the moisture.

Germination and seedling production tests in electrically-controlled ovens and in a greenhouse were made on the "fresh" seeds and after one, two, and three years of storage.

Eggplant and tomato seeds kept well in all conditions tried.

Carrot, lettuce, onion, and pepper seeds were injured by sealed storage at room temperature unless the seeds had been dried. At $-5°$ C. drying was not necessary. Dried sealed storage was superior to open storage at room temperature. Limited tests with cauliflower seeds indicated a similar response.

Lettuce seeds showed an increased power of seedling production in the soil with increased length of time in dry storage. This became very marked after three years of storage. Germination of these seeds, however, remained practically constant throughout the tests. Poor seedling production could be remedied by pre-treatment with light or low temperature.

Results of these tests to date indicate that open storage at room temperature in regions of the same general temperature and humidity as Yonkers, would effectively maintain vitality for two years in a majority of cases. However, if the moisture content of the seeds is reduced to approximately 6 per cent, and sealed containers are used, vitality is assured for at least three years. No drying is necessary if cold storage is used.

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FUNGICIDAL ACTION OF ORGANIC THIOCYANATES, RESORCINOL DERIVATIVES, AND OTHER ORGANIC COMPOUNDS[1]

FRANK WILCOXON AND S. E. A. MCCALLAN

The majority of fungicides which have found wide application are compounds of copper, mercury, or sulphur. Among organic compounds, with the exception of organic mercury derivatives, only formaldehyde has been used extensively, although numerous reports of investigations on others are found in the literature. While many organic substances including aldehydes, esters, acids, and phenolic compounds have been found to inhibit the germination of fungous spores, the concentration required is usually much higher than that of copper or mercury salts producing the same effect. Certain cases have been reported in which the natural resistance of the plant has been ascribed to the presence of small amounts of definite organic substances, toxic to the fungus. For example, evidence has been presented by Link, Walker, and coworkers (3, 10, 18) that the resistance of colored onions to smudge and neck rot organisms may be ascribed to the presence of protocatechuic acid and catechol in the outer scales. It was found that protocatechuic acid was capable of causing abnormal germination of the spores of *Colletotrichum circinans* in a concentration of 625 p.p.m., while this concentration of catechol entirely prevented germination (3, 10). Rokhlina (16) observed a correlation between resistance of the Cruciferae to *Plasmodiophora brassicae*, and the amount of volatile mustard oils formed from mustard oil glucosides in the case of the various species.

During a study of the value of certain organic thiocyanates as contact insecticides it was observed that these compounds also exhibited marked fungicidal action. The present paper presents the results of a laboratory study of the comparative toxicity of a number of organic thiocyanates, some related thiazoles, certain derivatives of resorcinol and other phenolic compounds. These organic compounds were compared with copper sulphate as a representative inorganic compound, the toxicity of which has been frequently studied, and with formaldehyde as the best known organic fungicide.

METHODS AND MATERIALS

Toxicity was determined by the method of spore germination previously employed and described (11, 12, 13). The spores are suspended in the solution to be tested and the percentage germination observed when there is no further change, that is, after about 20 hours. The conidia employed

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were those of the common American brown rot fungus, formerly cited as *Sclerotinia americana* (Worm.) Nort. & Ezek., but now generally considered to be *Sclerotinia fructicola* (Wint.) Rhem. (8, 15, 23), as well as those of *Botrytis paeoniae* Oud. and of *Pestalotia stellata* B. & C. A few supplementary tests were also made with the conidia of *Cladosporium fulvum* Cke. The first three species were grown on potato dextrose agar at 20° C. and the spores obtained from 6 to 9-day-old cultures in the case of *Sclerotinia fructicola*, and 6 to 15-day-old cultures with *Botrytis paeoniae* and *Pestalotia stellata*. To insure uniformly high germination in the controls filtered orange juice was added throughout so that the final concentration with *Sclerotinia fructicola* and *Botrytis paeoniae* was 0.02 per cent and with *Pestalotia stellata* 0.05 per cent (13, 21). The spores of *Cladosporium fulvum* were obtained from artificially inoculated tomato plants maintained in a humid chamber in the greenhouse.[2] The toxicity tests were performed at room temperature, the extremes of which were 21° C. and 27° C., with an average range per experiment of 22.4° to 24.9° C.

In testing a particular compound, at any one time, four concentrations were usually employed and each of these was tested in triplicate. About 400 spores were counted in a triplicate test of one concentration of one compound for one fungus. The tests with some of the more toxic compounds were repeated a number of times.

The criterion of toxicity is the LD 50, or dose permitting 50 per cent of the spores to germinate. The LD 50 may be obtained by plotting the logarithm of the concentration against percentage germination values transformed either to the "normal equivalent deviations" of Gaddum (6) as was done in a previous study (13) or to the more recent and fundamentally similar "probits" of Bliss (4). With either method the experimental results tend to be more or less on a straight line. Since the present paper is a preliminary survey, the LD 50 was estimated simply by drawing by eye the best straight line among the points and the precise method of fitting described by Bliss was not employed.

The preparation of the organic thiocyanates tested has been described in previous publications (9, 20). Certain thiocyanates readily undergo rearrangement to form thiazoles, which are heterocyclic ring compounds, no longer containing the SCN group as such (14, vol. 3, p. 141).

Methyl-thiazolone, C_4H_5NOS, was prepared from thiocyanoacetone by treatment with concentrated hydrochloric acid, as described by Hantzsch (7). The melting point was 102° C. The corresponding phenyl-thiazolone was prepared in a similar manner from thiocyanoacetophenone. It melted at 204° C. The so-called "thiocyanoacetic acid," C_3H_3NOS,

[2] The initial supply of infected leaves was kindly furnished by Dr. A. G. Newhall of Cornell University, Ithaca, New York.

which is also a thiazole derivative (14, vol. 1, p. 579), was obtained from methyl thiocyanoacetate. It melted at 122° C.

2-Methyl-4-*p*-chlorophenyl thiazole was obtained as the hydrobromide, as described by Wetherill and Hann (19) from thioacetamide and ω-bromo-*p*-chloroacetophenone. The product melted at 122° C.

ω-Thiocyano-*p*-chloroacetophenone was obtained from the above-mentioned bromide compound by refluxing with KSCN in alchool. It melted at 136° C. By treatment with concentrated HCl, a high melting thiazole derivative, presumably *p*-chlorophenyl thiazolone, was obtained, which melted with decomposition at 217°.

Benzothiazole guanidine and benzoxazole guanidine were obtained through the courtesy of Professor G. B. L. Smith of the Polytechnic Institute of Brooklyn, New York.

Thiodiphenylamine was obtained from Dr. R. C. Roark, United States Department of Agriculture, Washington, D. C.

Protocatechuic acid was prepared from Kino gum as described by Stenhouse (17).

Catechol, 2,4-dihydroxybenzoic acid and resorcinol were obtained from the Eastman Kodak Company, Rochester, New York.

Phenyl isothiocyanate was obtained from the Rubber Service Laboratories, Akron, Ohio.

Butyl, amyl, and hexyl resorcinols were prepared as described by Dohme, Cox, and Miller (5), by reduction of the corresponding acyl resorcinols.

Among the compounds listed are a number whose solubility in water is very slight. In these cases a solution was prepared by dissolving the compound in Penetrol (a proprietory miscible oil) and then diluting with water so that the most concentrated preparations contained 0.5 per cent Penetrol. As will be shown, Penetrol at this concentration had little or no fungicidal effect.

RESULTS

The results of the toxicity experiments with *Sclerotinia fructicola*, *Botrytis paeoniae*, and *Pestalotia stellata* are shown in Table I. The supplementary tests with *Cladosporium fulvum* indicated that the conidia of this fungus are approximately as sensitive to copper sulphate, phenacyl thiocyanate, and trimethylene thiocyanate as are the conidia of *Pestalotia stellata*. A number of compounds listed in the lower part of Table I showed little or no toxicity to this fungus.

From the point of view of possible application as fungicides, the compounds tested may be divided into two groups: the members of the first group shown in the upper portion of Table I have values of the LD 50 less than 100 p.p.m., while in the case of the less toxic compounds shown in the lower portion of Table I, the LD 50 exceeds this figure.

TABLE I

CONCENTRATIONS PERMITTING 50 PER CENT GERMINATION (LD 50), IN PARTS PER MILLION OF VARIOUS ORGANIC COMPOUNDS AND COPPER SULPHATE, LISTED IN ORDER OF AVERAGE TOXICITY

Compound	Approx. No. spores counted per fungus	*Sclerotinia fructicola*	*Botrytis paeoniae*	*Pestalotia stellata*
Copper sulphate	9000	5	4	5
Hexyl resorcinol	4400	7	18	5
Amyl resorcinol	5500	11	20	7
Phenacyl thiocyanate	5400	20	20	2
Ethylene thiocyanate	1000	20	—	—
Butyl resorcinol	8300	21	29	17
Thiocyano aniline	900	25	—	—
Butyryl resorcinol	1300	32	32	16
Trimethylene thiocyanate	11500	23	48	13
Methyl thiocyanoacetate	900	35	—	—
Thiocyanopropyl phenyl ether	1300	45	—	—
Phenyl isothiocyanate	1800	80	32	7
Lauryl thiocyanate	1850	63	>10	40
Thiocyanoacetate of diethylene glycol butyl ether	1200	93	80	22
Thiocyano methyl *p*-chlorphenyl ketone	690	>100	>100	—
2-Oxo-4-*p*-chlorphenyl thiazole	560	>100	>100	—
2-Methyl-4-*p*-chlorphenyl thiazole hydrobromide	750	>100	>100	—
Benzothiazole guanidine	820	>100	>100	—
Benzoxazole guanidine	270	>100	>100	—
Trimethylene dibromide	1150	160	—	—
Formaldehyde	3420	220	180	150
Methyl thiazolone	1780	250	250	—
2,4-Dinitrophenol	1340	290	250	—
2,4-Dihydroxybenzoic acid	1550	230	320	250
Ortho chlorophenol	3030	160	79	>1000
Ortho nitrophenol	1200	330	—	—
2-Oxy-4-phenylthiazole	1640	710	320	>100
Catechol	1790	250	140	1300
4-Oxy-thiazolone	3250	>1000	>1000	—
Resorcinol	1700	>1000	>1000	>1000
Protocatechuic acid	2330	>1000	>1000	>1000
Potassium thiocyanate	1200	>1000	>1000	>1000
Penetrol	1610	>5000	>5000	>5000
Thiodiphenylamine dust	430	Non-toxic	Non-toxic	Non-toxic

The organic thiocyanates in general show high toxicity, in some cases comparable to that of copper sulphate. The closely related thiazoles were much inferior to the thiocyanates in this respect. A recent patent (1) claims the use of thiazole derivatives as fungicides, but the thiazole compounds tested by us do not appear promising.

Another group showing high fungicidal efficiency is that of the acyl and alkyl derivatives of resorcinol. These results are in agreement with those of Woodward, Kingery, and Williams (22) who tested a large number of phenolic compounds on the three fungi *Monilia tropicolis*, *Cephalospor-*

ium sp., and *Sporotrichum* sp. and found such resorcinol derivatives to be extremely toxic.

On the other hand the toxicity of catechol and protocatechuic acid, considered by Link, Walker, and coworkers (3, 10, 18) to be the cause of resistance of certain onion varieties to smudge and neck rot organisms, was not particularly great. Woodward, Kingery, and Williams (22) found that catechol had a very low phenol coefficient, while Anderson (2) reported catechol and protocatechuic acid as comparatively ineffective in preventing the germination of uredospores of *Puccinia graminis tritici*.

Formaldehyde was less toxic than might be expected from its frequent use as a fungicide.

GREENHOUSE TESTS

Since the thiocyanates as a group proved highly toxic, several greenhouse tests of a preliminary nature were performed in an attempt to control tomato leaf mold. Comparisons were made of 0.1 per cent trimethylene dithiocyanate in Penetrol, 2.5 per cent and 10 per cent phenacyl thiocyanate dust in bentonite, 4-4-50 Bordeaux mixture, and 300 mesh sulphur dust. Four tomato plants (*Lycopersicon esculentum* Mill. var. Bonny Best) were sprayed or dusted with each compound to be tested. After the plants had dried they were inoculated by atomizing with a distilled water suspension of spores of *Cladosporium fulvum* Cke. The plants were held in an inoculation chamber for 48 hours and leaf spot counts taken two weeks later.

The Bordeaux and sulphur gave about 40 per cent control, as may be seen in Table II. The trimethylene thiocyanate was similarly effective though in some cases it caused a slight burning of the growing tips. The phenacyl thiocyanate caused such severe injury that leaf spot counts were impossible.

TABLE II

EXPERIMENTS ON THE CONTROL OF TOMATO LEAF MOLD IN THE GREENHOUSE. NUMBER OF SPOTS PER LEAF

Compound	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Average
0.1% trimethylene thiocyanate	24.8	6.9	4.1	1.7	9.4
300 mesh sulphur	25.2	6.2	6.0	3.3	10.2
4–4–50 Bordeaux	26.2	10.3	5.7	2.8	11.2
Control	32.3	14.5	10.5	11.1	17.1

SUMMARY

1. A laboratory study has been made of the toxicity of 32 organic compounds to the conidia of *Sclerotinia fructicola*, *Botrytis paeoniae*, and *Pestalotia stellata*. These compounds included organic thiocyanates, thiazoles, resorcinol derivatives, and other phenolic compounds, and were compared with copper sulphate and formaldehyde.

2. The thiocyanates and the alkyl and acyl resorcinols were found to be highly toxic, some members of these series approximating copper sulphate in effectiveness. Potassium thiocyanate, however, was non-toxic.

3. The thiazoles as well as catechol and protocatechuic acid were considerably less effective, the concentrations required to prevent germination being so high as to render their practical application unlikely.

4. Preliminary greenhouse experiments on the control of tomato leaf mold indicated that while trimethylene dithiocyanate was equal to Bordeaux mixture and sulphur dust none of these compounds gave satisfactory control of this disease.

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TESTING PLANT TISSUE FOR EMANATIONS CAUSING LEAF EPINASTY[1]

F. E. DENNY

In a previous paper (1) it was shown that when young potato plants were placed in closed containers with various types of plant tissue an epinasty of the potato leaf petioles similar to that caused by ethylene was induced.

The experiments have been continued, and a number of tissues have been added to the list of those capable of producing epinasty of potato leaves. Also, some tissues have been found which have failed to give the response.

The principal object of the present paper, however, is to suggest convenient methods of testing for plant-tissue emanations causing epinasty, and to show how apparatus ordinarily available may be utilized for class room demonstrations or for laboratory experiments.

SUGGESTIONS FOR APPARATUS

In order to reduce the volume of the air surrounding the leaves and thus to increase the concentration of any emanations which would be given off by the tissue being tested the method shown in Figure 1 A and 1 B was found satisfactory. Pieces of galvanized iron[2] were cut into the shapes shown in Figure 1 A, and by means of modelling clay a fairly tight joint around the potato stem and between the pieces of metal could be obtained. Bits of the plant tissue to be tested were placed on top of this metal shelf and the top of the potato plant was covered by the inverted glass beaker, the edge of which was then sealed with modelling clay. Figure 1 B shows the result obtained by enclosing with the potato plant fragments of leaves of rhubarb (*Rheum rhaponticum* L.).

Instead of using the entire potato plant with roots, the stem of the plant was cut off at the surface of the soil, or slightly below, and this cutting when placed in a vial of water was capable of giving the typical epinastic response (see 2, p. 317). The procedure shown in Figure 1 C was found to be satisfactory for most of the tissues tested. The plant cutting is inserted into a small bottle of water which is placed in the center of a large

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 100.

[2] Galvanized iron which has previously been in contact with vapors of ethylene chlorhydrin cannot be used for this purpose, since the metal itself after this treatment when placed in closed containers with potato plants caused epinasty of petioles. Zinc dust when moistened with ethylene chlorhydrin and allowed to dry spontaneously in air acquires the property of producing epinasty of potato leaves, presumably by the formation of small amounts of ethylene. The nature of the reaction between the zinc and the ethylene chlorhydrin, and of that by which ethylene is subsequently released, is being investigated.

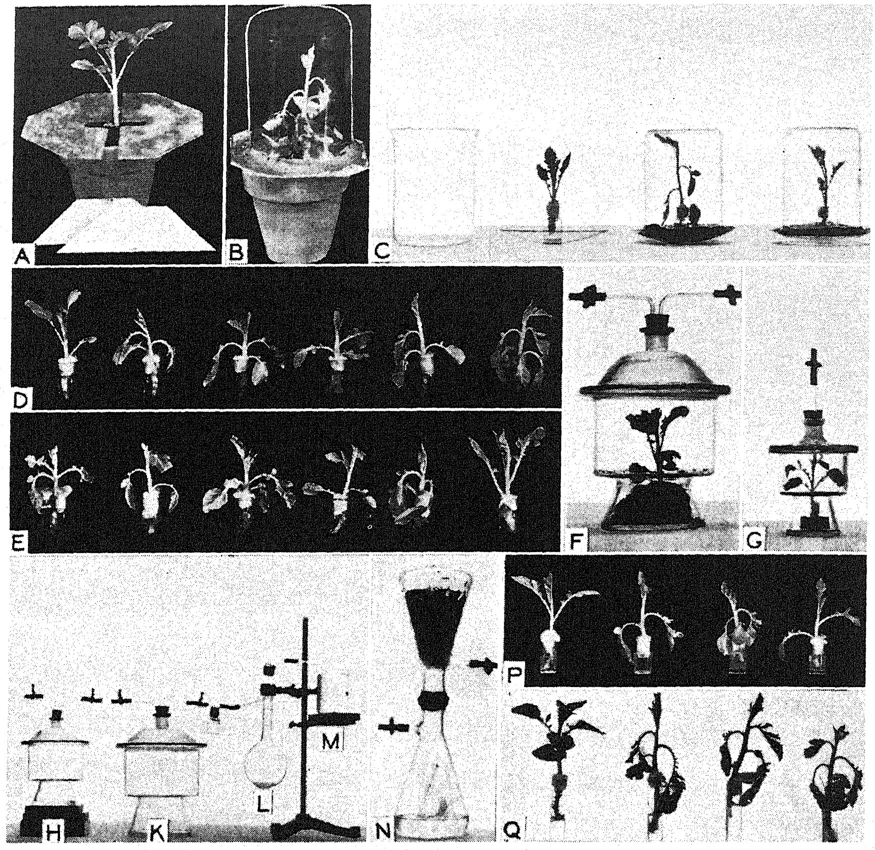

FIGURE 1. Apparatus, methods, and results in testing plant tissue for emanations causing leaf epinasty. A, metal shelf for enclosing top of potato plant with beaker. B, epinasty caused by enclosing 10 g. of rhubarb leaves under the beaker which covers the top of the potato plant. C, method of using tips of potato plants inserted in vials of water; right hand plant is the control, and plant in center shows epinasty caused by 5 g. of lettuce leaves placed in watch glass before sealing the inverted liter beaker. D and E, responses to 5 g. of various tissues in liter beakers; reading left to right: D, check, response to radish root, turnip root, beet root, rutabaga root, kohlrabi tuber; E, geranium petals, verbena petals, hollyhock pistils, rose pistils, spinach leaves, check. F, potato plant in soil in 3-l. desiccator which may be evacuated in order to introduce air previously in contact with plant tissue in closed containers. G, small desiccator (750 cc.) containing potato tip cutting in vial of water (procedure same as described for F). H to N, apparatus for accumulating plant tissue emanations over long periods and transferring the air to vessels containing potato test-plants (for details see text). P, effect obtained by method shown in C using 3 g. of chopped tissue in 18-l. bell jars, left to right: check, response to hollyhock pistils, petunia petals, lettuce leaves. Q, effect using apparatus shown in H to N, left to right: check, response to potato leaves, tomato leaves, peony stems.

watch glass. The tissue to be tested is scattered thinly on the watch glass and the beaker is inverted over the plant and the tissue. Modelling clay is applied as a seal at the edge of the beaker. The two plants at the right in Figure 1 C show the result of such a test, the plant at the extreme right being the check plant, and the center plant of the three showing the epinasty of potato leaves caused by bits of lettuce leaves. Further results by this method with various tissues are shown in Figure 1 D, 1 E, and 1 P.

Another procedure consists in placing the tissue that is to be tested in a desiccator or other closed container, and allowing an accumulation of the gaseous products. The air surrounding the tissue is subsequently transferred to another desiccator which contains the potato test plant. Figure 1 F (a 3-l. desiccator) and Figure 1 G (desiccator with a volume of about 750 cc.) show suitable types of containers. The potato plants, either with roots in soil as in Figure 1 F, or cut off and placed in a vial of water as in Figure 1 G, are placed in the desiccators which are then evacuated; they are then connected to the vessel containing the emanations accumulated from the tissue to be tested, and the air is transferred to the evacuated vessel, water being admitted to replace the air which is being transferred. By regulating the flow of air, by measuring the amount of water admitted, due regard being taken for the pressures reached at any stage, aliquot samples of the air can be transferred to the evacuated vessel.

When, in order to permit accumulation of emanations, the tissue was to remain in the closed containers for several hours or days provision was made to absorb the carbon dioxide formed and to replace the oxygen used in respiration. The apparatus shown in Figure 1 H to M was found satisfactory. A layer of N/1 KOH is placed in the bottom of desiccator K, and the tissue to be tested is placed in the upper part of K. As fast as oxygen is used for the respiration of the tissue in K it is drawn over from the flask L which was filled with pure O_2 at the start of the experiment. Reduction in pressure in flask L causes water to flow into L from the beaker M. The water in the beaker M is maintained at a constant level. In the present experiments this was done by a U-tube siphon from a water pipe (not shown) in which a flow of water at constant level was maintained. Desiccator H is not connected to desiccator K until the end of the period of accumulation. The potato test plant is then placed in H which is evacuated, and air from K is admitted to H by the method described in the preceding paragraph.

Instead of the desiccator K, the apparatus shown in Figure 1 N may be used. The upper filter flask is partly filled with bits of tissue (in the case shown, with pieces of the stem of peony) supported by a wad of cotton. The lower flask contains KOH solution with some pieces of glass tubing to increase the absorbing surface. The two filter flasks are separated by a gasket made from sheet cork, and a fairly firm joint can be made by winding with

gummed tape. A layer of modelling clay makes the joint air-tight. Figure 1 Q shows some results with tissues tested by this procedure.

TISSUES PRODUCING EPINASTY

In addition to the tissues described in the previous paper (1) the following have caused epinasty of potato petioles:

Petals. Petals of geranium (*Pelargonium hortorum* Bailey), verbena (*Verbena hybrida* Voss.), hollyhock (*Althaea rosea* Cav.), and petunia (*Petunia hybrida* Vilm.).

Anthers. Anthers of *Lilium davidii* Duch. removed from flower buds of which petals would not expand until the following days.

Pistils. Pistils obtained from unopened flowers of rose (*Rosa rugosa* Thunb.), squash (*Cucurbita pepo* var. *condensa* Bailey), and hollyhock (*Althaea rosea* Cav.).

Immature fruits. Fruits that were obviously immature and had not approached the period ordinarily referred to as the ripening stage from the following species: tomato (*Lycopersicon esculentum* Mill.), squash (*Cucurbita pepo* var. *condensa* Bailey), and apple (*Pyrus malus* L.).

Leaves. Leaves of lettuce (*Lactuca sativa* L.), rose (*Rosa rugosa* Thunb.), spinach (*Spinacia oleracea* L.), New Zealand spinach (*Tetragonia expansa* Murr.), onion (*Allium cepa* L.), Virginia creeper (*Parthenocissus quinquefolia* Planch.), potato (*Solanum tuberosum* L.), and tomato (*Lycopersicon esculentum* Mill.).

Roots. Roots of radish (*Raphanus sativus* L.), turnip (*Brassica rapa* L.), beet (*Beta vulgaris* L.), and rutabaga (*Brassica napobrassica* Mill.).

Tubers. Tubers of kohlrabi (*Brassica caulorapa* Pasq.).

The tissue was usually cut into small pieces with knife or scissors; the amount of tissue varied in different tests but was usually 5 g. per l. of air space in the container. In only a few cases was any attempt made to determine the lower limits of the quantity of tissue necessary to cause epinasty. Radish roots, immature fruits of squash and tomato, and lettuce leaves gave good responses with 2 g. of chopped tissue in an inverted 4-liter beaker. Pistils of hollyhock (with the tuft of styles removed), and petals of petunia, when cut into small pieces with knife or scissors, gave good responses when 3 g. were placed in 18-l. bell jars as shown in Figure 1 P. In later tests these two tissues gave distinctly positive tests for epinasty when only 1 g. of tissue was used in 18-l. bell jars.

Emphasis should not be placed on the exact quantities of tissue used in these experiments, however, since the response may depend upon the conditions preceding the time of sampling and during the period of testing. There was evidence, for example, that epinastic responses were obtained more readily in the summer than in the spring even with the same tissue. Temperature, length of day, age of tissue, or other factors, may influence

the production of emanations from the tissue, or the sensitivity of the potato test-plants, or both.

The time required for the development of the epinastic response after exposure to tissue or emanations from tissue varied with the tissue used; usually the bending of petioles began within about four to six hours. The observations were seldom continued beyond 24 to 30 hours from the start of the experiment.

Although, as a result of exposure to these plant-tissue emanations, the potato leaf petiole shows epinasty (downward bending), the lateral leaflets on the same leaf show hyponasty (upward bending). These small leaflets bend upward and tend to fold together on the upper side of the leaf. There is, thus, a curious contrast in the effect of the emanation on the cells in the petiole and on the cells of the leaflets which are attached to this petiole.

TISSUES NOT PRODUCING EPINASTY

Tubers of Irish potatoes (*Solanum tuberosum* L.), either whole or cut into pieces, and the germinated seeds of wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), or corn (*Zea mays* L.), were placed in containers such as K in Figure 1 and allowed to remain in the container for 48 hours, oxygen being supplied meanwhile from the flask L in Figure 1. When the air surrounding these tissues was transferred to desiccators, such as H in Figure 1, containing young potato plants, the epinastic response was either negative or inconclusive.

Using the same apparatus, negative results were obtained from the fermentation of sugar with baker's yeast, and from the growth of *Rhizopus nigricans* Ehr. in test tubes with potato dextrose agar (2 per cent) as substrate.[3]

The fully expanded pileus and stalk of two species of mushrooms, one being *Russula emetica* Fr., and the other not definitely identified but believed to be a species of *Lepiota*, when placed under inverted beakers with potato plants by the method shown in Figure 1 C, failed to induce epinasty.

CARBON DIOXIDE AS A FACTOR

In many of the tests a watch glass or evaporating dish containing N/1 KOH was enclosed in the container in order to absorb the carbon dioxide as fast as it was formed. Tissues which produced epinasty in the absence of KOH also produced it when the CO_2 was absorbed by KOH. In the experiments in which the apparatus shown in Figure 1 H to N was used the CO_2 was continuously absorbed by the KOH solution in the vessel. Attempts to produce epinasty with carbon dioxide at concentrations of 5, 10, 20, 40, and 80 per cent by volume were unsuccessful, and this was true

[3] Thanks are extended to Keith O'Leary for preparing the fungus cultures in suitable condition for the experiment.

whether the CO_2 was obtained from a cylinder of the compressed gas as purchased commercially, or when generated in the laboratory from C.P. sodium bicarbonate and sulphuric acid.

It was found that a sufficiently high concentration of CO_2 interfered with the epinastic response of potato plants to tissues that cause epinasty in the absence of CO_2 or in the presence of only small amounts. Ten grams of squash tissue in a 3-l. desiccator caused strong epinasty of potato leaves; the simultaneous addition of CO_2 did not interfere with the response to the squash tissue until the CO_2 concentration reached 40 per cent; this amount reduced perceptibly the epinastic response, and at 80 per cent CO_2 epinasty was not obtained. The epinasty is a growth response and presumably these higher concentrations of CO_2 interfere with the growth of the plant and consequently with its ability to show epinasty of leaves.

TESTS WITH ESSENTIAL OILS

Essential oils[4] from 41 different species of plants were tested for their effect with respect to epinasty. Pieces of filter paper 40×5 mm. to which were added two drops of the oil were placed in 7-l. closed containers with test plants. In some cases the oils were diluted with alcohol in order to avoid injury to the plants. Epinasty of tomato leaves did not occur in any case after 48 hours' exposure. Tests of 14 of these volatile oils with potato plants were likewise negative after 24 hours.

These preliminary tests with essential oils are as yet not entirely conclusive. The oils of the various plant species used in the experimental tests with tissues were not available, and not enough tests have been made as to the effect of the concentration of oil vapors; but these negative results indicate that it is unlikely that the epinasty is caused by volatile oils from the tissues.

SUMMARY

Apparatus and details of procedures for testing plant tissues for the production of emanations which cause epinasty of potato leaves are described.

Epinasty was produced by volatile products from 16 different species of plants in addition to those previously reported upon. The plant organs giving positive responses included petals, anthers, pistils, immature fruits, leaves, and tubers.

Tissues failing to produce epinasty were potato tubers, germinating seeds of corn, wheat, and oats, the actively growing mycelium of *Rhizopus nigricans*, and the mature sporophores of two species of mushrooms.

[4] Our appreciation is expressed for the cooperation of Dr. E. K. Nelson of the United States Department of Agriculture in furnishing these samples of essential oils.

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TOBACCO AS A TEST PLANT FOR COMPARING THE EFFECTIVENESS OF PREPARATIONS CONTAINING GROWTH SUBSTANCES

A. E. Hitchcock

In a previous report (3) methods were described for determining the relative effectiveness of preparations containing synthetic growth substances in causing certain formative responses such as bending, swelling, proliferation, and rooting on tomato, tobacco, and several other plants. These responses were shown to be essentially the same as those induced by carbon monoxide, ethylene, acetylene, and propylene. Although quantitative measurements were made of the epinastic (downward bending) response induced by preparations containing these growth substances, the details of the measurements were not given. It is the purpose of the present paper to give the results of these measurements and to show that the tobacco plant may also be used to test the effectiveness of similar preparations in causing rooting or in retarding or stimulating the growth of axillary buds following removal of the stem tip.

Treatment of one leaf on a tobacco plant with a lanolin preparation containing a high concentration of growth substance causes bending only on the test leaf, and does not induce measurable bending on any other leaf. In contrast to this type of local response, the tomato represents a class of plants which shows systemic bending when treated in a similar manner—that is, more than one leaf may show a measurable bending response. It is thus not feasible to apply more than one treatment to a single tomato plant, whereas on the tobacco as many treatments may be made as there are leaves which can be measured. Although less sensitive than the tomato, the tobacco has a number of advantages which make it more desirable as a test plant than the tomato, particularly for the quantitative measurement of the epinastic response.

MATERIALS USED

The substances used in these experiments were indoleacetic acid (hetero-auxine), indolepropionic acid, phenylacrylic acid, and phenylpropionic acid, which were previously used (3), and indolebutyric acid, phenylacetic acid, and naphthaleneacetic acid, which have been recently reported by Zimmerman and Wilcoxon (5). These substances were mixed with anhydrous lanolin, olive oil, or with a preparation containing either one-half lanolin and one-half Amalie oil (a commercial mineral oil), or three-fourths lanolin and one-fourth Amalie oil. Lanolin preparations were used in most of the bending and rooting tests. The ingredients for lanolin preparations were weighed out and mixed in small glass vials hav-

ing a capacity of 10 to 20 cc. Thorough and quick mixing was accomplished by immersing the lower half of the vial for a few seconds in water ranging in temperature from 30° to 55° C., the main purpose of this procedure being to melt the lanolin. In addition to the crystalline acids mentioned above, ethylene and propylene were also taken up in lanolin.

The tobacco (*Nicotiana tabacum* L. var. Turkish) was the principal test plant used in these experiments.

METHODS

Epinasty. Preparations of the growth substance were applied with a small glass rod to the upper side of the base of the petiole over a distance of approximately one-half inch. Measurements of the angle between the leaf and the portion of the stem above were made at the time of treatment and on each of four successive days. The increase in this angle due to treatment was used as the criterion of the relative effectiveness of the different preparations in causing epinasty.

Distribution of the treatments was according to the Latin Square method (2, p. 234). Seven different growth substances were applied to each tobacco plant, but a different concentration was used on each plant. The number of plants used in a single test, therefore, corresponded to the number of concentrations used. Generally four or five concentrations were used, and each test was replicated three or four times. Thus, for example, in one test (Fig. 1) five concentrations of each of seven substances were applied to five plants in such a manner that the same concentration of all substances appeared on the same plant. Since, in addition, a lanolin control preparation was applied to one leaf on each plant, the total number of treatments was 40. This particular test was replicated four times, so that 160 preparations were applied on 20 tobacco plants. Although a given substance appeared at a different level (leaf) on each plant, the same order was maintained on all plants—that is, phenylacrylic acid, for example, always appeared below indolebutryic acid, except when the former appeared at the top of the series. The same concentration of each growth substance appeared at four different levels on four different plants. The plants were from 15 to 20 inches in height at the time of treatment.

Rooting. The same preparations were used for the rooting tests as were used for the bending tests, and the method of application and the arrangement of the treatments on the tobacco were essentially the same, except that the stem internodes were treated instead of the petioles of leaves. Lanolin preparations of the growth substances were applied half way around the stem over a distance of approximately three-fourths of an inch. Each successive treatment was placed off of the vertical axis so that it would not lie in a direct line with adjacent treatments above or below. The plants were from 15 to 20 inches in height at the time of treatment.

Bud growth. The tips of tobacco plants were cut off and a preparation of growth substance was applied to the cut surface. One series of plants was from 20 to 30 inches in height and the other series was from 6 to 8 inches in height at the time of treatment. In no case had the axillary buds shown any signs of growth when the first treatment was made. The tall plants were treated with preparations containing the crystalline acids and the short plants were treated, in addition, with lanolin containing ethylene or propylene. In preparing the lanolin-gas mixtures, several grams of lanolin were smeared on the inner walls of a salt-mouth bottle from 500 to 1000 cc. capacity. The bottle was then filled with water and the water displaced with the gas. These bottles were then stored at 15 degrees centigrade until the preparation was used.

RESULTS

MEASUREMENT OF THE EPINASTIC RESPONSE

The average change in angle of tobacco leaves receiving different treatments is shown for tests A and B in Figure 1. Each point represents the average angle of declination for four leaves. These two sets of curves were constructed so as to show the actual direction of movement made by the leaves. In order to do this it was necessary to change the zero point and reverse the abscissa and ordinate values as compared to the normal manner which is shown in Figure 2. In the active bending range, an increase in concentration of three to three and one-half times caused an increase in declination, except for high concentrations of phenylacrylic and phenylpropionic acids. The degree of declination produced by a given growth substance and the relative effectiveness of the different substances are seen to be nearly the same in both series of tests, even though the tests were run at different times. These and other relationships are more readily seen if the concentrations required to produce different degrees of bending are plotted as shown in Figure 2. In this case average values for tests A and B shown in Figure 1 were used to construct the curves in Figure 2.

The curves in Figure 2 show that indole preparations are more effective than phenyl preparations in causing epinasty of tobacco leaves. Indoleacetic acid was more effective than indolepropionic acid and phenylacetic acid was more effective than phenylpropionic acid. For the lower concentration range (1 to 3 mg. per gram of lanolin) indolebutyric acid is seen to be slightly more effective than indolepropionic acid. Average values for higher concentrations were not available, and hence the curve in this range is based on a single test and is shown by a broad dashed line. Average values for the phenylacrylic and phenylpropionic curves were likewise not available for all concentrations.

It was stated previously (3) that olive oil and certain other oils were more efficient carriers than lanolin for indoleacetic, indolepropionic, phenyl-

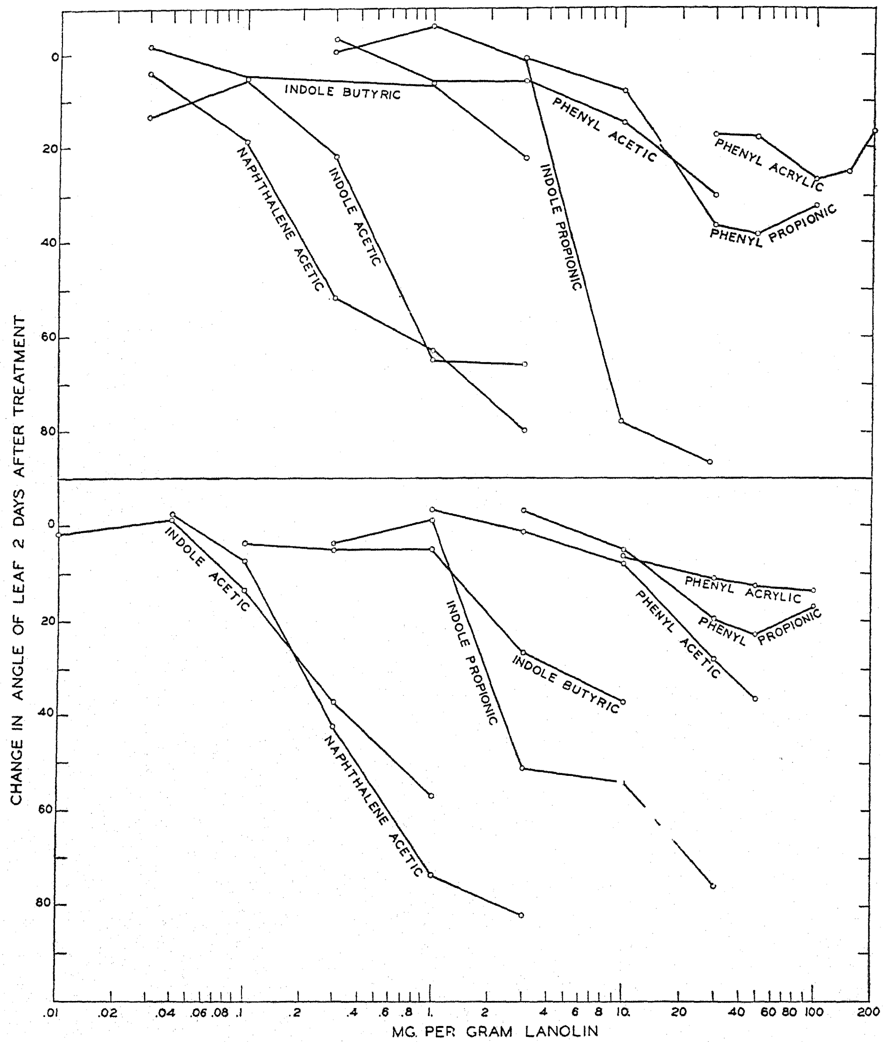

FIGURE 1. Relative effectiveness of lanolin preparations containing different growth substances in causing epinasty of Turkish tobacco leaves. Plotted so as to show actual direction of leaf movement. Average values for these two series of tests are plotted in the usual manner in Figure 2. Tests A (above) and B (below) were performed at different times.

acrylic, and phenylpropionic acids. Curves for lanolin and olive oil preparations which contained the same amount of indolepropionic acid are shown as test C in Figure 2 by a dotted line and dot-dash line respectively.

The lanolin curve (dotted line) follows very closely the curve based on the more recent tests and shown by the solid line. The corresponding curve for the olive oil preparation (dot-dash line) shows an activity from 6 to 8 times that of the lanolin preparation. In fact, the olive oil preparation of

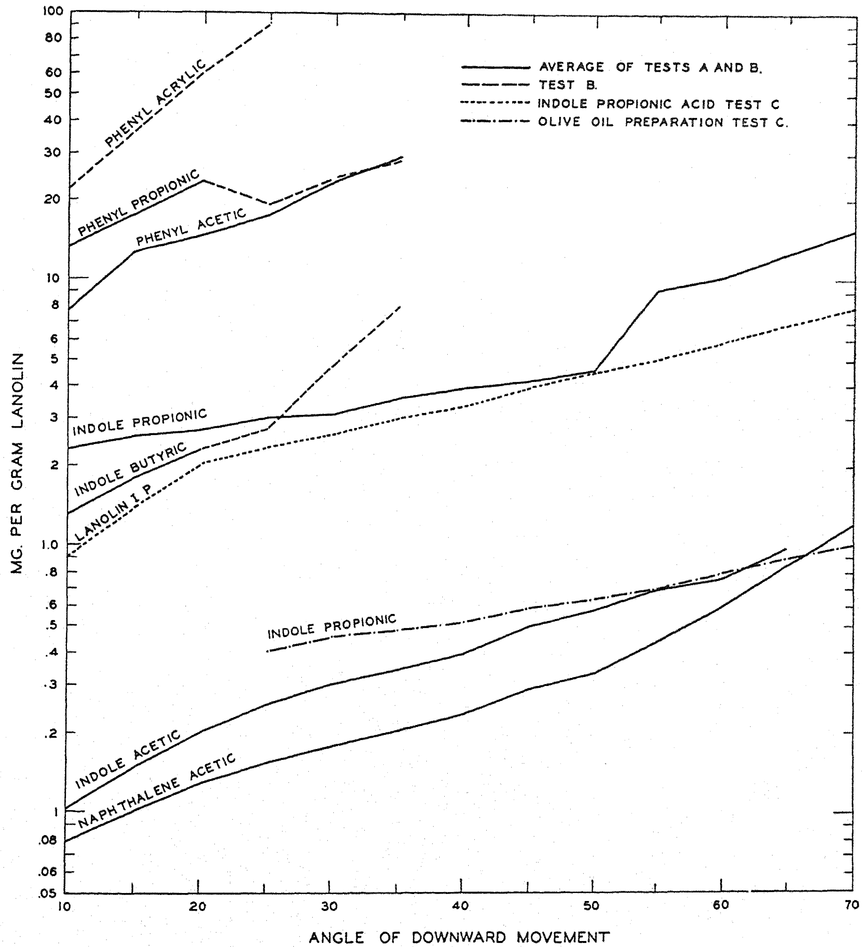

FIGURE 2. Relationship between concentration of growth substance and the epinastic response of Turkish tobacco leaves. Data calculated from curves in Figure 1 for tests A and B, and from curves for test C which are not shown.

indolepropionic acid shows nearly the same activity as the lanolin preparation of indoleacetic acid.

The effectiveness of the different substances in causing epinasty of tobacco leaves may be judged not only from the standpoint of the minimum active concentrations causing epinasty, but also from the standpoint

of the range of concentrations over which the epinastic response occurs. In this respect, naphthaleneacetic and indoleacetic acids were much more effective than the other five acids (Figs. 1 and 2). Phenylacrylic and phenylpropionic acids were the least effective. Phenylpropionic acid, for example, increased in effectiveness from 10 to 50 milligrams per gram of lanolin, which represents a maximum range of five times. Indoleacetic and naphthaleneacetic acids showed a corresponding increase of 10 to 30 times. Other tests for which curves are not given indicate that the latter two substances increase in bending effectiveness from 0.1 up to at least 50 milligrams per gram of lanolin which represents a range of 500-fold. Higher concentrations produced bending, but the amount of the increase in declination was not proportional to the increase in concentration, and there was usually some injury accompanying the response. The range for indolepropionic acid was approximately 50-fold. The curve for indoleacetic acid shows the closest relationship with respect to the increases in declination caused by an increase in concentration, particularly over the range 10 to 40 degrees. The control lanolin preparations caused only a slight declination, the average values for the several tests ranging from 0.8 to 1.4 degrees. Leaves receiving no treatment showed an average declination of 1 degree.

On the basis that indoleacetic acid showed the closest relationship between concentration and the amount of declination, the corresponding or equivalent amounts of the other substances required to produce the same degree of bending were calculated from the curves in Figure 2. These calculated values are shown in Table 1. Since values for a declination greater

TABLE I

CONCENTRATIONS REQUIRED TO INDUCE A GIVEN DEGREE OF DECLINATION ON TOBACCO LEAVES, AS CALCULATED FROM THE DATA IN FIGURE 2

Angle of declination	Relative amount of growth substance calculated to induce the same amount of declination as indoleacetic acid					
	Naphthaleneacetic	Indolepropionic	Indolebutyric	Phenylacetic	Phenylpropionic	Phenylacrylic
10	0.80	23.5	13.0	76	130	220
15	0.67	17.0	11.7	83	117	233
20	0.63	13.8	11.8	72	115	300
25	0.60	12.0	11.0	70	78	360
30	0.58	10.3	14.7	77	80	
35	0.57	10.3	22.8	91	83	
40	0.55	9.4				
45	0.58	8.6				
50	0.55	8.1				
55	0.64	11.9				
60	0.79	13.4				
Av.	0.63	12.6	14.2	78	107	278

than 30 degrees were not available for all substances, average values are shown in order to give an approximation of the probable relationship for bending up to 60 degrees. Also, these average values were used as a guide in making up equivalent concentrations that were calculated to produce a 30-degree declination. The results of these tests are shown in Table II.

TABLE II

EPINASTY OF TOBACCO LEAVES INDUCED BY CONCENTRATIONS CALCULATED FROM DATA IN FIGURE 2 TO PRODUCE A 30-DEGREE DECLINATION

Growth substance	Concentration mg. per g.	Average angle of declination for 6 leaves
Indoleacetic acid	0.3	26
Phenylacrylic acid	84.4	24
Indolebutyric acid	4.8	78
Indolepropionic acid	3.6	27
Phenylacetic acid	23.4	56
Naphthaleneacetic acid	0.2	30
Phenylpropionic acid	30.0	41

The results in Table II show that five of the seven calculated concentrations induced an angle of declination which approximated 30 degrees. The bending values for indolebutyric and phenylacetic acids were too high. All of the calculated concentrations in Table II were not exactly proportional to the average values shown in Table I. This is due to the fact that some of the preparations already made up were of nearly the same concentration as the calculated values, so that an additional adjustment was not considered necessary. The fact, however, that five of the seven concentrations produced bending that approximated 30 degrees is no doubt purely accidental. The important point in this type of test is that equivalent concentrations should produce approximately the same amount of bending and not necessarily a given degree of declination. Since the degree of declination for indolebutyric acid was nearly three times that for indolepropionic acid, it seemed evident that in this particulr concentration range indolebutyric acid was more effective than indolepropionic acid. This fact is of course substantiated by the curves for these two substances shown in Figure 2, but since in the higher concentration range indolebutyric acid appeared to be less active than indolepropionic acid, it was felt that the average values for equivalent concentrations shown in Table I might be more nearly correct. In order to verify these results, an additional test was made in the lower concentration range (1 to 5 mg.) for these two growth substances. The results of this test are shown in Table III.

The relative differences in bending for indolebutyric and indolepropionic acids shown in Table III are in agreement with the data in Figure 2. It must be concluded, therefore, that in the range from 1 to 5 mg. per gram of lanolin, indolebutyric acid is slightly more effective than indolepropionic

acid in causing epinasty. The results of this test also show that relatively small differences in concentration induce measurable differences in declination of the leaves.

TABLE III

COMPARATIVE EFFECTIVENESS OF LOW CONCENTRATIONS OF INDOLEBUTYRIC AND INDOLEPROPIONIC ACIDS IN CAUSING EPINASTY ON TOBACCO LEAVES

Concentration of growth substance mg. per g.	Average angle of declination for four leaves	
	Indolebutyric acid	Indolepropionic acid
1.00	38	—
1.50	47	31
2.00	63	—
2.25	—	46
3.00	68	66
4.50	—	73

ROOTING TESTS

The concentrations used for the rooting tests were as follows: 0.1, 0.3, 1.0, 3, and 10 milligrams per gram of lanolin for indoleacetic, indolebutyric, and naphthaleneacetic acids; 3, 10, 30, 50, and 100 for phenylacetic and phenylpropionic acids; 0.3, 1, 3, 10, and 30 for indolepropionic acid; and 10, 30, 50, 100, and 150 for phenylacrylic acid. The total concentration range for all substances was therefore 1500 times. The order of treatments from top to bottom on the first plant was phenylacetic, indolepropionic, naphthaleneacetic, phenylpropionic, lanolin control, indoleacetic, indolebutyric, and phenylacrylic acids. Although each growth substance appeared at a different level (internode) on different plants, the order of the substances was the same on each plant as in the case of the bending tests. Phenylacrylic acid, for example, was always applied below indolebutyric acid except when the former appeared on the uppermost of the treated leaves. In the first series of tests 160 preparations were applied to the stems of 20 tobacco plants. Each concentration of each of the seven growth substances was applied at a different level on four different plants. Two days later the same treatments were applied to 20 tobacco plants that had more succulent stems than those in the first series.

Results of these tests showed that the rooting response on tobacco stems varied according to the concentration of growth substance, the kind of substance, and the location of the treatment along the vertical axis. Rooting was induced by the three highest concentrations of all growth substances. Phenylacetic and phenylacrylic acids were the only two substances for which rooting was induced by the lowest concentration used in these tests. The next lowest concentration of all substances except indolepropionic acid caused rooting. The total number of treatments which caused rooting was 46 for the highest concentration, and 42, 38, 18, and

4 respectively for the next lowest concentrations. These are the average values for the two series of tests in which 160 treatments were applied to 20 plants in each of the two series. Each treatment was therefore replicated eight times.

Rooting induced by the highest concentrations was delayed on some of the plants or in some cases was accompanied by visible injury such as discoloration or a marked increase in diameter of the roots. Indolebutyric and indoleacetic acids were the least injurious in the high concentrations. From the standpoint of a good rooting response, indolebutyric acid was the most effective, followed in decreasing order by indoleacetic and phenylacrylic, phenylacetic, naphthaleneacetic, phenylpropionic, and indolepropionic acids. If judged from the standpoint of the total number of treatments which induced rooting, the decreasing order of effectiveness was as follows: phenylacetic and phenylacrylic acids, naphthaleneacetic, indolebutyric, phenylpropionic, indoleacetic, and indolepropionic acids.

Preparations which induced bending of the stem, as illustrated in Figure 3, were usually very effective in producing roots. The plant on the right was treated with the highest concentrations of the seven growth substances, and the plant on the left with the lowest concentrations of the same materials. After several weeks roots usually appeared from tissue adjacent to the treated areas in the case of the most effective treatments. The plant on the right in Figure 3 illustrates this type of response. The extent to which one treatment will affect other nearby treatments will therefore depend to a certain degree upon the duration of the experiment. During a period of two weeks this effect was not noticeable.

Treated internodes on the same plant varied in their capacity to form roots. The total number of rooted internodes from top to base was as follows: 16, 22, 25, 24, 23, 16, 14, and 7. These results show that the portion of the tobacco stem beginning from one to two inches below the tip and extending down for four to six inches, roots more readily than the very young tissue near the tip or the older tissue on the lower half of the stem. It was observed that the same treatments were equally effective farther down on succulent stems as compared to stems of less actively growing plants. The most pronounced rooting responses occurred on the second and third internodes.

BUD GROWTH AFTER DECAPITATION OF STEM TIP

When tips of tobacco stems were cut off and the cut surface was treated with a control lanolin preparation, one or more of the upper three buds commenced growth. Generally at least two shoots developed to a length of several inches, and then one of these assumed leadership and the other one ceased growth. There was no growth of side buds on control tobacco

plants which were not decapitated, and there was no evidence of flower bud formation at the time these treatments were begun.

FIGURE 3. Rooting response on stem of Turkish tobacco after 53 days. Lowest concentrations of seven different growth substances applied separately and to different internodes of plant on left. Highest concentrations of same growth substances applied to plant on right. Leaves removed at time of photographing.

If the cut surface of decapitated stems was treated with a lanolin preparation containing a high concentration of any one of the seven growth substances used in the bending tests, marked inhibition of the growth of

the upper two or three buds occurred. The degree of inhibition was proportional to the concentration of the substance. In the case of indoleacetic

FIGURE 4. Bud growth after decapitation of stem tip. A. Control plants after 31 days. Three plants on left not decapitated; three on right decapitated and control lanolin preparation applied to cut surface. B. Lanolin-Amalie oil preparation of indolepropionic acid (50 mg. per g.) applied to cut surface after decapitation.

and indolepropionic acids, noticeable inhibition resulted from treatment with preparations containing from 3 to 100 mg. per gram of lanolin. No attempt was made to compare the effectiveness of the different substances in these tests. In addition to inhibiting the growth of some of the upper buds, these same preparations induced the growth of many of the buds lower down the stem as illustrated in Figure 4 B. One of the most effective preparations contained 50 milligrams of indolepropionic acid per gram of a mixture consisting of equal parts of lanolin and Amalie oil.

FIGURE 5. Young tobacco plants 20 days after decapitation. Lanolin preparations containing the following substances were applied to the cut surface, from left to right: none (control), indolepropionic acid, ethylene, and propylene.

As a general rule, at least three of the buds below the second one and above the eleventh bud, developed into shoots that were several inches in length after a period of 20 to 30 days. In one case, for example, the third, seventh, and ninth nodes produced shoots 10, 6, and 16 inches in length, respectively. In another case the fourth, eighth, and tenth buds developed into shoots 4, 5, and 7 inches long, respectively. Eventually, however, one of the upper shoots became the leader and the other shoots ceased to elongate. In addition to the shoots which attained a length of several inches, there were others which formed one or more leaves from one to three inches

long but failed to exceed a stem length of one inch. Shoots of this type are shown in Figure 4 B. All of the plants in this series of tests were from 20 to 30 inches in height at the time of treatment.

Lanolin preparations containing ethylene or propylene were tested in a similar manner on young actively growing tobacco plants from 6 to 8 inches in height. Essentially the same response resulted from treatment with the lanolin-gas mixture as with the preparations containing the crystalline acids (Fig. 5). When the lanolin-gas mixture was applied on alternate days until four applications had been given, the response was much more pronounced than when only one or two applications were given. In these tests ethylene was more effective than propylene. These results appear to be similar to those previously reported for the forcing effect of ethylene gas on latent rose buds (4) in which it was shown that 70 per cent of all buds on a treated plant formed shoots.

DISCUSSION

The results of tests relating to the epinastic response of tobacco leaves showed that the declination caused by the crystalline growth substances is a measurable response. Differences in concentration of one and one-half to three and one-half times produced measurable differences in the degree of declination of the leaves. The fact that the bending response of one leaf on a tobacco plant is independent of other treated leaves on the same plant, makes it possible to apply several treatments to a single plant. Thus a large number of preparations may be tested on a relatively small number of plants. In preliminary tests it was found that the capacity of leaves to bend downward varied with the age of the leaf. The degree of declination decreased with increasing age of the leaf. Variations due to the age of the leaf and to individual differences among plants were compensated for when the distribution of treatments was made according to the Latin Square method. Four replicates of each treatment proved sufficient to demonstrate measurable differences in the epinastic response. Each of the four replicate treatments, for example, was applied to a different level on a different plant.

Turkish tobacco was selected as a test plant primarily because many comparisons could be made with the use of a small number of plants. The results obtained with tobacco will not necessarily be the same as those resulting from the use of another kind of test plant. It is believed, however, that the order of relative effectiveness of the different growth substances in causing epinasty will be approximately the same for other test plants such as the tomato, African marigold, and sunflower. With respect to the minimum active concentration, there will no doubt be differences according to the kind of test plant used. The minimum concentration of indoleacetic acid which caused epinasty on tomato leaves was 0.003 mg. per gram of

lanolin, whereas for tobacco the minimum concentration was approximately 0.1 mg. (Fig. 2). The same stock preparation of naphthaleneacetic acid used in these tests was slightly more effective than indoleacetic acid on tobacco (Fig. 2), but the minimum active concentration for the tomato was between 0.03 and 0.1 mg. or the same as for the tobacco. These results are similar to those reported for the relative effectiveness of ethylene and certain other gases in causing epinasty on different test plants (1). Additional data on the minimum active concentrations of the different growth substances are given by Zimmerman and Wilcoxon (5).

The relative effectiveness of the different growth substances depends not only upon the kind of test plant used, but also upon the carrier or solvent. Olive oil and certain other oils were reported as more effective carriers than lanolin (3). The quantitative difference for an olive oil preparation of indolepropionic acid is shown in Figure 2. The use of water as a solvent for the growth substances would also be expected to give different results. Lanolin has been used as the principal carrier because of the ease with which the preparation may be applied to the plant and because it does not appear to injure the plant. All lanolin preparations were made with anhydrous lanolin.

Tests performed at different times with the same preparations did not produce the same degree of bending, but the order of relative effectiveness of the different growth substances was essentially the same (Fig. 1). For this reason comparisons should be made simultaneously or under standard conditions with respect to atmospheric conditions, the age and activity of the test plants, and the age of the preparations used. After having determined the relative effectiveness of several growth substances, one of these may then be used in all future tests as a standard of comparison. If, for example, standard preparations of indoleacetic acid are used in all tests, the relative effectiveness of other preparations will be on a comparable basis. Any loss in activity of a preparation of growth substance could be readily detected by the method described. This would apply more particularly to water preparations of the growth substances. The method used for injecting water preparations has been described in an earlier report (3).

In the rooting tests it was evident that a pronounced and uniform rooting response was limited to the upper part of the stem, and hence approximately half of the treatments in these tests were placed on tissue which was not capable of exhibiting an optimum rooting response. The tobacco is therefore less efficient as a test plant for rooting than it is for the bending response. It is believed that, if the treatments are applied only to the portion of the stem which is capable of bending, more accurate comparisons could be made. This would of course necessitate the use of fewer treatments per plant.

In will be noted that the order of relative effectiveness in rooting was

not the same as for bending. No doubt the relative effectiveness of the different growth substances in causing rooting will vary according to the kind of plant to which they are applied. Certain unpublished data relating to the treatment of shoots of hardwood plants would appear to support this idea. Indolepropionic acid, for example, was more effective than the other six acids in causing rooting of the Japanese maple. In similar tests with the Grimes Golden apple, indoleacetic was more effective than indolepropionic acid, and these were the only two of the growth substances tested which induced rooting. Diphenylacetic acid which caused rooting on the African marigold but not on the tomato failed to induce epinasty on either plant. For this reason, diphenylacetic acid has not been reported as an active growth substance.

Tests with the decapitated tobacco plants showed that apical dominance was interfered with by the application of growth substances to the cut surface. The irregularity of shoot growth below the cut surface of treated plants indicated that there was no uniform influence exerted by the buds or subsequently formed shoots on the upper part of the stem as was the case on the control plants. The fact that a lanolin preparation of ethylene caused the same type of response is a further indication of the similarities previously mentioned (3) between the crystalline growth substances and the gases. In some of the bending tests, the lanolin preparations containing growth substance were sometimes applied in such a manner that the axillary bud was covered. It was observed that in the case of the high concentrations the axillary buds started growth and then stopped after reaching a length of one-fourth to three-eighths of an inch. The fact that latent buds were induced to grow as a result of treatment directly or indirectly with crystalline or gaseous growth substances suggests the possibility that other types of dormant buds may undergo changes of a similar nature when their dormancy is broken naturally or by artificial means. Decapitation of the rapidly growing canes of the Crimson Rambler rose and the application of the lanolin preparations of growth substance to the cut surface resulted in responses that were essentially the same as those induced on the tobacco. In this case, however, the high concentrations prevented the growth of all buds on canes that were three to five feet long. Slightly lower concentrations induced the growth of three shoots near the cut surface, but not necessarily the first three. At the end of seven weeks these shoots had attained a length of one to three feet. Much lower concentrations acted the same as the control lanolin preparation. On control canes a shoot grew from the first node and in a few cases also from the second node. Experiments with decapitated plants are presented primarily to show that the Turkish tobacco may prove useful as a test plant for determining the effects of growth substances on bud growth, as well as for the epinastic and rooting responses.

SUMMARY

1. Five concentrations of each of seven different growth substances were tested simultaneously and at different times on leaves of Turkish tobacco in order to determine the relative effectiveness of the preparations in causing epinasty. The decreasing order of effectiveness was as follows: naphthaleneacetic and indoleacetic acids, indolebutyric and indolepropionic acids, phenylacetic, phenylpropionic, and phenylacrylic acids.

2. The relative effectiveness of the same preparations was also determined for rooting on stems of the tobacco. From the standpoint of a good rooting response the decreasing order of effectiveness was as follows: indolebutyric acid, indoleacetic and phenylacrylic acids, phenylacetic, naphthaleneacetic, phenylpropionic, and indolepropionic acids. Since there were marked differences in the capacity of different portions of the stem to form roots, the order of effectiveness given represents the average response of internodes at different levels and is not based on the optimum rooting response for each substance.

3. Application of lanolin preparations of growth substance to the cut surface of decapitated tobacco stems retarded or inhibited the growth of the upper buds and stimulated the growth of many buds on the middle and lower part of the stem. Although the normal apical dominance was disturbed for a period of several weeks, eventually one of the upper shoots became the leader. Lanolin preparations of ethylene and propylene caused similar responses.

4. Tobacco was found to be better adapted for testing the epinastic response than it was for the rooting response.

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EFFICIENCY OF BANDING FOR THE CONTROL OF CANKERWORMS

ALBERT HARTZELL AND W. J. YOUDEN

In 1933 cankerworms had practically defoliated a tract of woodland in Yonkers, New York, belonging to the Institute. Since control by spraying was not considered feasible because of the rocky nature of the terrain, which made the operation of a high pressure sprayer difficult and expensive, it was decided to band a section of the woodland to determine if this method would give sufficient control to warrant the cost of banding of the whole tract with the view of preventing defoliation in future outbreaks. Accordingly, the trees in a section that was somewhat isolated were banded in the fall of 1933. The failure of this method to give any appreciable control in the severe outbreak that occurred during the spring of 1934 suggested a study of the efficiency of banding and a search for the cause that made this method ineffective.

Porter and Alden (5) have published recently a comprehensive investigation on cankerworms and reviewed the literature. As a knowledge of some of the details of the life history and habits of these insects is important for a proper understanding of the problem involved, they are briefly reviewed here.

DESCRIPTION AND SEASONAL HISTORY OF CANKERWORMS

Cankerworms are among our oldest native pests and have been known in northeastern United States for more than two centuries. The larvae (Fig. 1 B) are called inch-worms, measuring-worms, span-worms, or loopers, because of their size and peculiar looping habit of locomotion. In Europe the term cankerworm is used to designate a number of different species of caterpillars. In the United States the term is restricted to two species, the fall cankerworm (*Alsophila pometaria* Harris), and the spring cankerworm (*Paleacrita vernata* Peck). These two species have much in common and their larvae often appear simultaneously. The same control measures have been applied generally to both. Outbreaks have occurred in cyclic periods averaging about 20 years apart during the last century in the vicinity of New York. In the Mississippi Valley practically all records refer to the spring species (*Paleacrita vernata*). Both species are said to prefer elm and apple but also feed on a wide range of host plants including many deciduous fruit and forest trees, particularly species of Rosaceae. The larvae defoliate the trees leaving only midribs and the larger veins with a few ragged shreds of tissue (Fig. 1 D). In lighter infestations the leaves are not entirely consumed but turn brown and dry, giving the appearance of having been swept by fire.

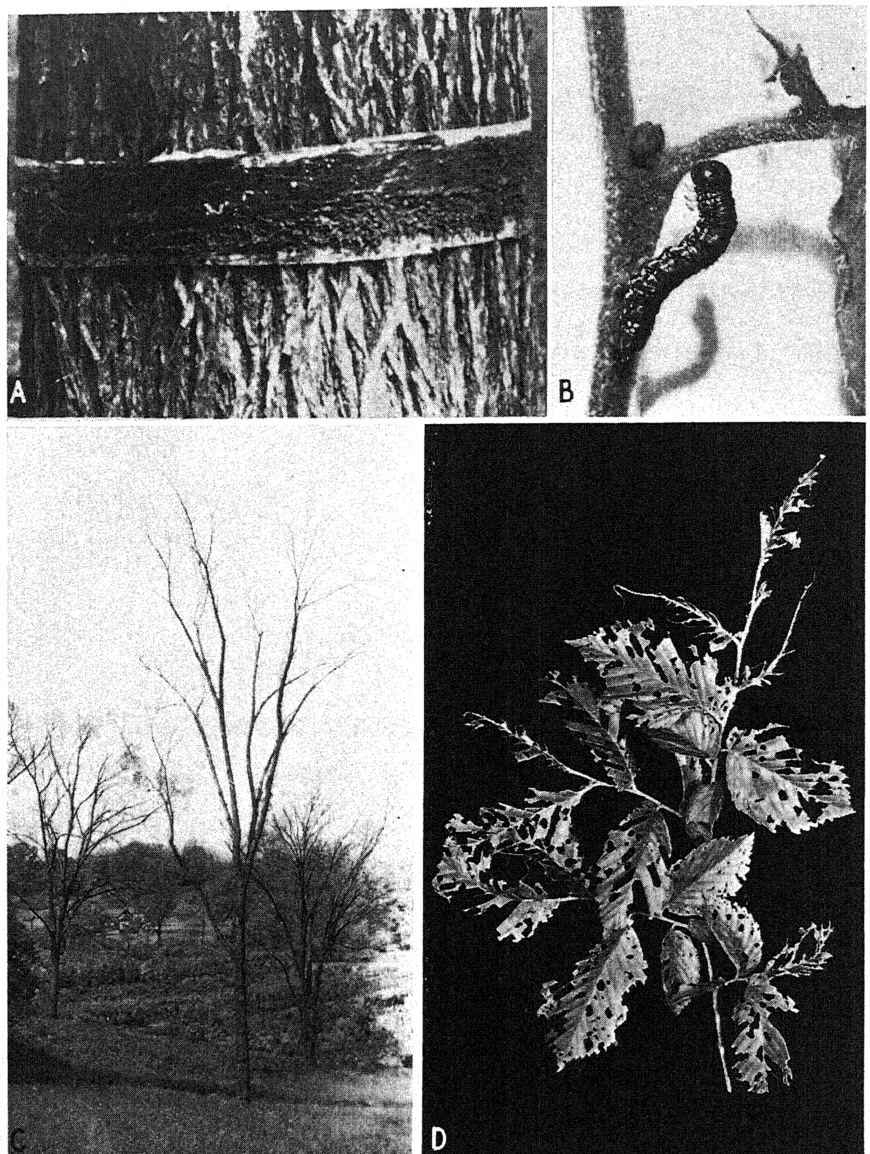

FIGURE 1. A. Banded trees showing type of band used for control of cankerworms. B. Partly grown cankerworm larva. × 3.7. C. Isolated American elm trees in foreground that were defoliated by cankerworms although banded. D. Injury caused by cankerworm larvae to foliage of American elm.

FALL CANKERWORM

The eggs are laid in the fall by the wingless adult females in masses containing a hundred or more. They are deposited in straight rows and resemble miniature flower pots. The egg masses which are ashy gray in color, while often observed on the trunks of trees, are found in greater numbers on the smaller branches near the tips. The females are 6 to 10 mm. in length and like their eggs are ashy gray in color. The eggs hatch early in May in the vicinity of Yonkers, New York, at the time apple blossoms show pink. Hatching extends over a period of from 4 to 12 days in a given locality. The newly hatched larvae make their way to the unfolding leaves and buds and commence feeding. At first they gnaw small pits into either surface of the leaf but they soon make large irregular holes until finally only the midribs and larger veins remain.

When disturbed, the larvae drop, supported by silken threads. The caterpillars complete feeding in 4 to 6 weeks. According to Porter and Alden (5) they enter the ground during the period extending from the first to the third week in June, at the close of the fourth instar. They burrow to a depth of about two inches, depending upon the kind of soil, and spin their cocoons.

Any time during the fall, but especially after freezing weather has occurred, the moths emerge. Upon emergence the females make their way to near-by trees and begin to climb the trunks. In very cold weather the ascent may take place over several days. Females are usually found in the field in advance of the males, which are winged. The adults are sluggish during the day but become active at dusk at which time they mate. There is a predominance of females over males. The moths lay their eggs over a period of about three weeks. There is one generation a year.

SPRING CANKERWORM

The moths emerge early in the spring very soon after the frost is out of the ground. They may occasionally leave the ground during warm periods in winter. The habits of the moths are similar to those of the fall cankerworm described above. The female moths (which are wingless) climb the trunks of the trees. In from 2 to 6 days they lay their eggs and continue to oviposit for a period of about 10 days. The eggs are deposited in concealed places, to some extent on the trunk and larger branches, but mostly on the smaller branches and the tips of the twigs. They are oval and just before hatching are almost black. The larvae of this species have a tendency to remain on the twigs which they closely resemble, while the larvae of the fall cankerworm, normally green in color, stay on the leaves. The feeding period extends to the end of the second week in June.

The fifth instar larvae enter the ground during May and June but unlike the fall species do not form cocoons. There is a single generation a year.

BANDING EXPERIMENTS

A tract of woodland (Fig. 2) in the Institute Arboretum consisting principally of a mature stand of American elm (*Ulmus americana* L.), red maple (*Acer rubrum* L.), black birch (*Betula lenta* L.), and hickory (*Carya ovata* [Mill.] K. Koch) was banded using the method described in the following section. Approximately 100 trees were banded. A roadway on the north and east sides served as a barrier. On the south side a 50-ft. clearing separated this tract from the main portion of the woodland. The land slopes steeply to the west toward the Arboretum nursery which had been sprayed the previous season with lead arsenate (5 lbs. to 150 gal.) and was sprayed each season that the experiment was in progress. This tended to keep the cankerworm population from building up and entering the banded tract.

The bands were placed on the trees October 23, 1933. A second coat of tanglefoot was applied during the last week of November when the moths were observed climbing the trees. Tanglefoot was applied again on May 1, 1934, to prevent the female moths of the spring species from ascending the trees. The trees were in full leaf by June 28.

On October 9 and 10, 1934, tanglefoot was applied to the bands. The female cankerworms were observed ascending the trees during the week of November 4 to 10, 1934. Again in the spring of 1935 tanglefoot was applied as soon as the ground had thawed.

The 1934 outbreak, which was the most severe and defoliated the greater part of the area, consisted chiefly of the fall cankerworm while in 1935 both the fall and spring species were present. In this investigation no attempt was made to determine the relative amount of injury caused by each species.

METHODS

Banding. A strip of cotton batting two or three inches wide was placed around the trunk of the tree at a height of four feet. This was covered with a band of asphalt building paper four inches wide, drawn tight and tacked so that the ends overlapped. Finally, a uniform coat of tree tanglefoot about one quarter of an inch thick was applied to the paper by means of a wooden paddle. The tanglefoot covered about two-thirds the width of the band (Fig. 1 A). The cotton batting served to fill any irregularities in the bark and prevented the moths from passing beneath the paper band. The asphalt paper was of high quality, threaded with one-half inch squares, which prevented it from being torn in handling or on exposure to weather. The tanglefoot and bands were renewed from time to time to prevent the moths from climbing over the bodies of entangled individuals.

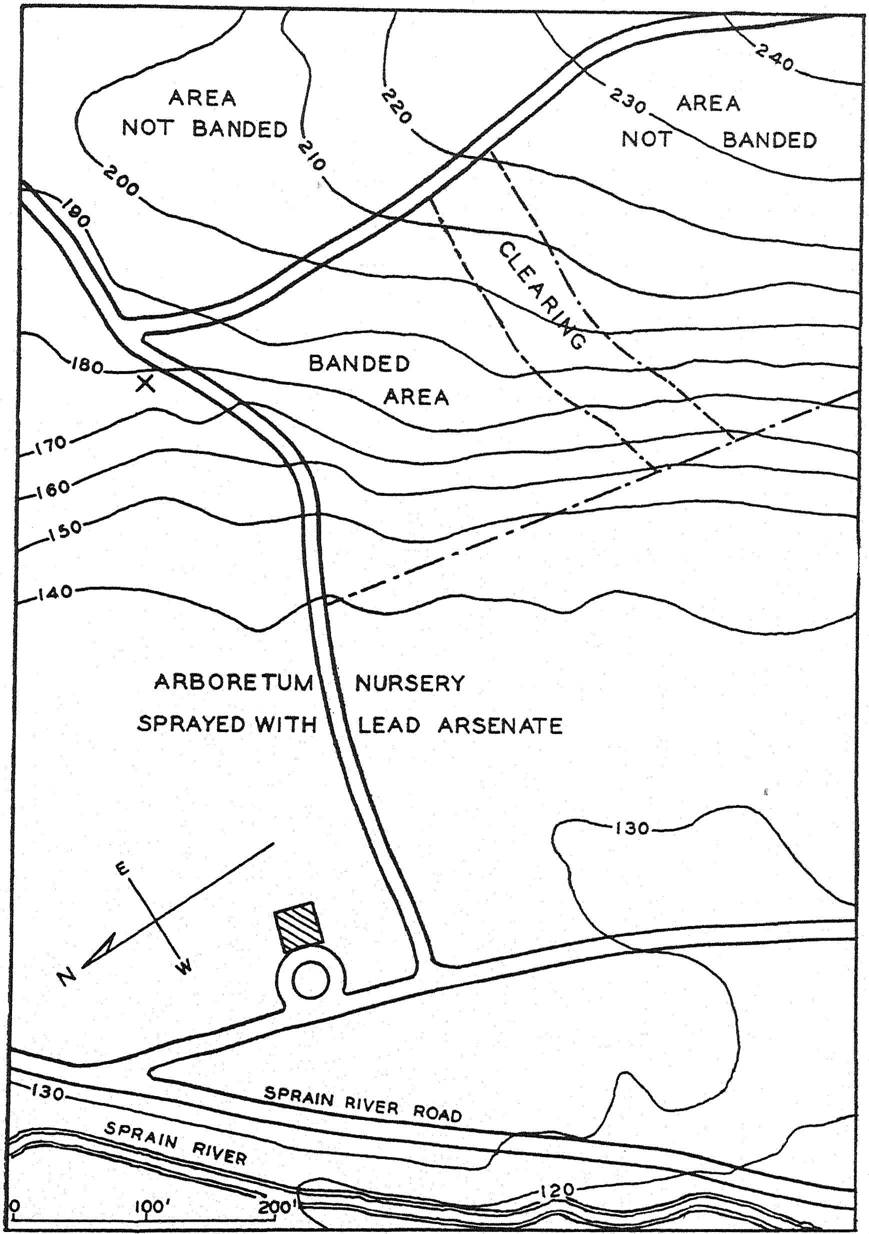

FIGURE 2. Map of tract of woodland banded for control of cankerworms. X marks location of apparatus for catching cankerworm larvae drifting with the wind.

Sampling. In order to evaluate the effect of banding, a method was devised for measuring the destructive action of the cankerworms upon the leaves of the trees. Inspection of the trees disclosed great variation in the amount of damage, so that it was necessary to make the samples as extensive as the time, labor, and trees available permitted. It followed that some rapid estimate of leaf damage to a large number of leaves was more important than precise estimates of a few samples. Accordingly, a tentative procedure was adopted for the work in 1934 and the results used as a basis for modifications introduced the following year. A number of banded trees and an equal number of unbanded trees of corresponding species were chosen at random. In 1934 two branches were cut from each tree and three samples of 25 leaves selected from each branch, making a total of 150 leaves from each tree. The following year, four branches were taken at random from different parts of each tree and two samples of 25 leaves picked from each branch so that 200 leaves were obtained. The 25 leaves were selected by cutting from a branch a number of small sections bearing only a few leaves and picking *every* leaf from the small sections until a total of 25 was reached. The sample of 25 was treated as a unit and its weight in grams recorded. In order to test this procedure, two workers each took two branches and turned the samples over to a third worker for weighing. By comparing the weights of the totals of 100 leaves picked by each man from each tree, the efficiency of the sampling technique was readily disclosed. Since the comparison of banded and unbanded samples involves different trees, the variation among trees is the critical factor. It is important, however, that the error of evaluating each tree should not be greater than that arising through the unavoidable variation among the trees; and while it is desirable to have it somewhat less, it is needless to go to the trouble of making it much less. The method outlined above proved to be entirely adequate for evaluating a tree so that the final accuracy depended solely on the inherent variation among the trees and the number of trees sampled.

RESULTS

While banding has been recommended for the control of cankerworms in this country for nearly a century (3, p. 336), there seems to be little or no information available on the efficiency of this means of control.

The results of the 1934 work are given in condensed form in Table I which shows the average weights of 150 leaves from trees sampled on four different dates. Frequently, a tree was resampled on a subsequent day so that although 80 trees were sampled that year, only 43 different trees were involved. For all species over all sampling dates, the leaves from the banded trees showed an average weight about 11 per cent greater than

the unbanded specimens and an examination of individual tree variation showed this not to be significant for the number of trees surveyed.

TABLE I

SUMMARY 1934 DATA; AVERAGE WEIGHT IN GRAMS OF 150 LEAVES

Species	No. trees	Banded	June 5	June 6	June 7	June 8	Av.
Elm	3	Yes No	25.8 28.6	20.6 20.2	16.8 35.6	19.7 22.5	20.7 26.7
Maple	3	Yes No	46.0 52.1	45.7 23.0	55.5 33.8	30.5 21.6	44.4 32.6
Birch	2	Yes No	25.0 30.5	25.3 13.9	32.7 24.6	28.2 20.4	27.8 22.3
Cherry	1	Yes No	32.6 30.0	38.9 36.5	26.3 21.5	26.6 23.5	31.1 27.9
Hickory	1	Yes No	34.4 32.8	41.6 33.4	30.1 23.5	33.8 29.9	35.0 29.9
All trees	10	Yes No	33.2 36.6	33.0 22.7	33.9 30.3	26.7 22.7	31.7 28.1

Total number of different trees sampled = 43

Table II gives the results in some detail obtained from the American elms in 1935. The weights of the individual samples of 25 leaves are not indicated since they are of interest only in showing variation within a branch and between branches of the same tree. Table II does show clearly that two individuals can sample a tree much more closely than the variation between different trees. The overall averages for the banded and unbanded leaves differ by about seven per cent, which is well within the expected limits of variation. The averages for the two workers were 20.8 and 19.8 grams, which, considering that the individual leaves varied from being completely intact to a destruction leaving only the midrib and veins, shows how successfully the samples were selected.

The summary of the 1935 data given in Table III shows for each day the average weights of 100 leaves obtained by each worker for both the banded and unbanded trees for the five species included. The number of trees of any given species varied on the different dates as indicated in the table. Table III shows the close agreement of the average weights from the banded and unbanded lots except in the two cases of cherry and hickory. Lack of specimens of these species in the tract under study prevented the use of adequate numbers to give satisfactory averages. It does not seem necessary to present detailed statistics computed from the data recorded since the day-to-day fluctuation in the results indicates that there is no

TABLE II

WEIGHT IN GRAMS OF 100 LEAVES FROM AMERICAN ELMS, 1935

Banded Tree No.	Date and individual taking sample								Av.
	May 24		May 29		June 5		June 12		
	Y*	H*	Y	H	Y	H	Y	H	
102	21.3	22.2							
106	20.8	18.5	20.2	20.3					
108	33.5	39.1			29.8	27.7			
110	15.0	14.4					11.6	13.0	
122			33.8	39.9	21.9	25.4	19.8	24.0	
125			25.5	26.9			26.1	25.6	
126			13.7	16.6			15.2	18.1	
142					14.7	16.0			
144					13.6	13.7	15.9	14.1	
161							28.3	25.7	
164							17.8	21.5	
166							17.0	18.2	
169							15.9	15.0	
Individ. Av.	22.6	23.5	23.3	25.9	20.0	20.7	18.6	19.5	
Av.	23.1		24.6		20.4		19.0		21.1
Unbanded Tree No.									
112	21.7	21.7			15.6	15.6			
116	37.0	34.6							
118	12.6	13.5	14.8	18.6			15.1	14.7	
120	17.3	13.8	18.8	19.7			17.8	17.9	
132			16.0	17.0					
135			23.4	22.1					
151					15.6	13.4			
152					9.5	13.4			
154					26.2	23.9	28.4	34.4	
172							10.6	13.1	
174							18.5	19.6	
175							25.5	31.2	
176							21.5	19.9	
178							11.4	11.7	
179							24.2	29.9	
Individ. Av.	22.1	20.9	18.3	19.4	16.7	16.6	19.2	21.4	
Av.	21.5		18.8		16.7		20.3		19.6
Av. for H		22.2		22.6		18.6		20.4	20.8
Av. for Y	22.4		20.8		18.4		18.9		19.8

* Y and H are the initials of persons taking separate samples of the same material.

consistent difference in favor of the banded trees. It is possible that by greatly extending the number of trees a difference might be shown. The data at hand lead to the conclusion that for the elms in this tract during these two years of severe infestation, the difference in average leaf weight resulting from banding is less than ten per cent.

TABLE III

SUMMARY 1935 DATA; AVERAGE WEIGHT IN GRAMS OF 100 LEAVES

Date	May 24		May 29		June 5		June 12		Av.	
Sampled by	Y*	H*	Y	H	Y	H	Y	H	Y	H
No. elms	4	4	4	4	4	4	9	9	21	21
Wt. banded	22.6	23.5	23.3	25.9	20.0	20.7	18.6	19.5	20.6	21.7
Wt. unbanded	22.1	20.9	18.3	19.4	16.7	16.6	19.2	21.4	19.1	20.0
No. of maples	4	4	5	5	3	3			12	12
Wt. banded	32.5	31.4	34.1	35.1	32.6	37.0			33.2	34.4
Wt. unbanded	29.1	25.4	37.7	39.2	21.8	27.9			30.8	31.8
No. birch	1	1	1	1	1	1			3	3
Wt. banded	17.1	14.5	18.0	18.1	19.2	22.8			18.1	18.5
Wt. unbanded	19.9	21.1	21.3	21.2	19.3	24.4			20.2	22.2
No. cherry					1	1			1	1
Wt. banded					20.5	18.9			20.5	18.9
Wt. unbanded					13.6	14.9			13.6	14.9
No. hickory					1	1			1	1
Wt. banded					38.5	35.7			38.5	35.7
Wt. unbanded					27.3	27.1			27.3	27.1
Total number of different trees sampled = 49										

* Y and H are the initials of persons taking separate samples of the same material.

WIND DISPERSION A DETERMINING FACTOR IN CONTROL

The phenomenon of wind dispersion of caterpillars was conclusively demonstrated more than 20 years ago by Collins (1) with gipsy moth larvae (*Porthetria dispar* L.). He records that newly hatched living larvae were caught on tanglefoot screens on the Isles of Shoals from a point on the mainland 13.5 miles distant. The maximum distance that this writer (2) found that these larvae were dispersed by the wind was from 19 to 30 miles across Cape Cod Bay. In summarizing the results obtained in wind dispersion of gipsy moth larvae at different stations, he states that the larvae of three other species of Lepidoptera, and possibly of a fourth species, were windborne for distances ranging from 200 feet to two-thirds of a mile. Among these were two small larvae of an unidentified species of Geometridae, the family to which the cankerworms belong. They were caught on a tanglefoot screen placed at a distance of from 200 to 500 ft. from the nearest tree growth. These larvae were practically nude and not clothed with hairs bearing swellings or vesicles, which are believed to have a buoyant action, as in the case of the gipsy moth larvae.

In the present investigation, cankerworm larvae occurred on banded trees in numbers sufficient to defoliate them in spite of the fact that neither in the fall nor spring were any female moths observed that had succeeded in crawling over the bands. This suggested that the larvae had infested

the trees by some other means than by hatching from eggs laid above the bands. As observed by Porter and Alden (5) the larvae, by letting themselves down from the limbs on strands of silk, may be blown from one tree to another. The writers did not determine, however, the extent of infestation by this means.

In order to determine the possibility of wind drift, eight shields covered with tanglefoot and directed toward the cardinal and intercardinal points of the compass were erected on a pole 20 feet high just north of the banded area. From time to time the shields were examined for the presence of cankerworm larvae and the tanglefoot renewed when needed. A tanglefoot barrier was maintained on the pole to prevent any larvae from climbing up from the ground. The site selected was in a cleared space where no larvae could reach it except by passing through an air space of at least 25 ft. To the west, the land slopes away rapidly and there were no infested trees in direct line of the shields for about 500 ft.

DESCRIPTION OF APPARATUS

Figure 3 A and B shows two views of the apparatus constructed for detection of wind dissemination of cankerworm larvae. It is constructed on the principle of a well-sweep to enable the shields to be brought down so that they can be examined by one standing on the ground. By loosening two set screws, the shields may be rotated to bring the eight shields alternately in a plane to be examined for the presence of larvae in the tanglefoot. Important specifications for the construction of this apparatus are listed as follows:

Three in. galvanized standard weight pipe, 20 ft. long.

Pivoted in center (10 ft. from ground) between two other 3 in. galvanized pipes 13 ft. long set in concrete 3 ft. deep in ground.

Eight shields at top 2 ft. wide $\times$ 3 ft. long, made of No. 16 gauge galvanized sheet steel. All edges turned 1/2 in. at 90° angle.

Angles supporting wings 3/16 in. $\times$ 1-1/2 in. $\times$ 6 ft., bolted to cast iron spider fitted to top of 3 in. pivoted pipe. Spider swiveled to permit turning for examination of wings.

Balance weight of shaft inside pipe 185 lbs.

RESULTS

Larvae of both species were captured on the shields from May 18 to June 12. The larvae were removed from the shields with forceps on each examination, after which new tanglefoot was applied. A number of larvae were alive when removed. Individuals up to one-half an inch in length were caught. That the larvae did not drift in from the nearest available source is indicated by the fact that some were taken on all shields. A total of 99 larvae were caught on a combined area of 48 sq. ft. and removed

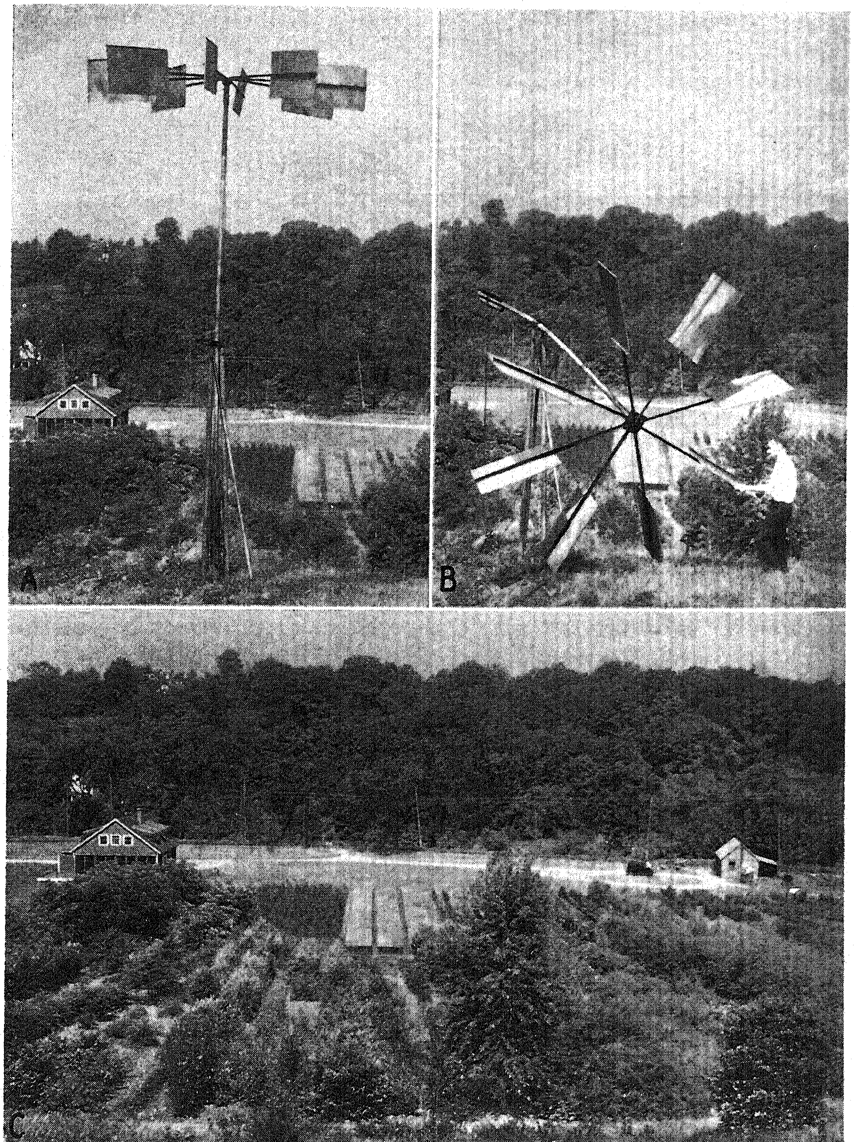

FIGURE 3. A. Apparatus consisting of 8 stationary shields covered with tanglefoot for catching cankerworm larvae drifting with the wind. B. Apparatus tilted for observing cankerworm larvae caught on the tanglefoot shields. C. View across valley looking west from wind-drift apparatus. From this point the nearest infested trees were 500 ft. distant.

from the shields throughout the season of 1935. Detailed results appear in Table IV.

TABLE IV

NUMBER OF CANKERWORMS FOUND ON TANGLEFOOT SHIELDS ON DIFFERENT DATES FACING GIVEN DIRECTION

Date, 1935	N	NE	E	SE	S	SW	W	NW	Total
May 18	2	1	2	3	2	0	3	0	13
May 24	1	8	3	5	0	1	0	1	19
May 28	5	5	4	5	2	3	0	5	29
June 3	0	8	2	5	1	0	4	7	27
June 12	5	1	2	1	0	0	0	2	11
Total	13	23	13	19	5	4	7	15	99

The last line of Table IV shows the totals for the eight direction shields. As a group, these totals show more variation than might be expected through chance alone. The four shields facing the northwest, north, northeast, and east have a total of 64 larvae, or nearly twice that on the other four shields. This is a significant concentration of the counts indicating that the direction is a factor in the distribution of the larvae. Reference to wind records (4) for this vicinity show that the prevailing and high velocity wind movements during this period of time came from the directions faced by the shields with the high counts. The average wind velocity for the period of May 18 to 31 was 8.8 miles per hour. The maximum velocity was 27 miles per hour on May 29. While the prevailing winds were from the northwest, winds of high velocity from the northeast the latter part of May, during the time the larval population reached its peak, account for the proportionally larger number of captures on the shields facing this direction. A heavily infested wooded area lay in the path of the winds blowing from this point of the compass. Toward the west and northwest there were no infested trees (Fig. 3 C) nearer than 500 ft., hence the corresponding shields caught fewer larvae.

Assuming that the density of the larvae per cu. ft. of air space was the same at all heights above ground as that surrounding the shields, in an air space occupied by a tree 60 ft. high and 20 ft. wide, approximately 5000 larvae would have drifted during the period of May 18 to June 12 when these observations were recorded.

DISCUSSION

As the larvae have strands of silk streaming from them by which they become attached to the branches and foliage when dispersed by the wind, it is readily seen how it is possible for trees to become infested during periods of high wind velocity. Since most of the larvae would drift above the bands, it is not surprising that banding has definite limitations.

While it is possible that an isolated tract of woodland could be protected efficiently by banding, isolated banded trees at distances of 50 to 100 ft. from the border of the banded tract were completely defoliated (Fig. 1 C).

With lighter infestation, it is possible that the bands would have been more effective.

SUMMARY

An attempt to control severe outbreaks of cankerworms during the seasons of 1934 and 1935 by banding resulted negatively.

An evaluation of the efficiency of banding showed a difference of less than 10 per cent between the average weights of samples of leaves from banded and unbanded trees.

It was demonstrated by means of captures on tanglefoot shields that cankerworm larvae are disseminated by wind. The failure of banding is believed to be due to this factor.

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STORAGE OF SOME CONIFEROUS SEEDS

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INTRODUCTION

The length of life of seeds of various kinds has been a subject of interest from early times. Turner (33) in his review of literature on the subject says that Theophrastus mentions that "Of seeds some have more vitality than others as to keeping" and "in Cappadocia at a place called Petra, they say that seed remains even for forty years fertile and fit for sowing." Turner also cites the work of de Candolle who in 1832 observed that when certain portions of soil were exposed to the air after several centuries, they became covered the first year with certain species, sometimes uncommon in the vicinity. He concluded that the seeds of these species had remained viable for considerable periods in the soil.

In 1879 Beal buried seeds of a number of different species of weeds in the soil in bottles filled with moist sand and made germination tests at intervals after burial. He (6) found seven kinds still alive after 30 years. Darlington (13) reporting on the 50-year period for Dr. Beal's experiment found that five species were still alive.

Until comparatively recently, however, the articles appearing on the subject of vitality of seeds have had insufficient data on which to base any real conclusions. With the perfection of reliable methods of seed testing the problem of seed storage has had a new impetus and at the present time many experiments are being conducted along this line. Results from many of these experiments have been reported in part or in full. Different workers have considered temperature, moisture, gas supply, light, vacuum, or a combination of two or more of these factors, in the establishment of good storage conditions for various seeds.

A considerable amount of work has been done on the vitality of coniferous seeds. Cieslar (9) in 1897 found that sealed storage of *Picea excelsa*, *Pinus austriaca*, and *Pinus sylvestris* lengthened the life of the seed. The difference in germination of seeds from sealed and open storage after six years was found to be as high as 33 per cent. Haack (20) confirmed some of the results of Cieslar. He found that sealed as against open storage reduced the fall in germination power of pine seeds 16 to 68 per cent in different samples in three years. He also found that the seeds must be dried thoroughly if they are to be stored at moderately high temperatures. Drying, however, had no effect on sealed storage in a cellar or an unheated room. He found open storage to be possible only in a dry room. He also emphasized the necessity of a distinction between germinating percentage and the percentage of actual plant production. He found

that as the germination percentage rose the power to produce plants rose more rapidly. This rise in germinating percentage gave an even greater rise in seedling production when the conditions were bad in the seed or in the seedling bed. He emphasized the fact that only quick growing seeds produced plants in the seed bed. Coker (11) referred to the remarkable retention of vitality in still unopened cones in *Pinus attenuata* and some other forms and the presence of good seeds in 20- to 30-year-old cones. He further pointed out that the conditions existing in serotinous cones were almost ideal for the preservation of seed vitality because of the regulation of gas exchange and humidity. Tillotson (31) working on *Pinus ponderosa*, *P. monticola*, *P. strobus*, *P. contorta*, *Picea engelmanni*, and *Pseudotsuga taxifolia* spread all seeds out thinly on a floor and fanned steadily for two days with an electric fan after which he stored them under various conditions. He also found that storage in air-tight containers was far superior to any other. In this condition seeds were little affected by temperature. However, contrary to findings of other workers he reported the poorest results from low temperature storage.

In 1928 an anonymous article (26) on the keeping quality of Noble fir seed (*Abies nobilis*) reported the beneficial effect of cold storage. These seeds were well dried before sealing in air-tight glass jars.

Wakeley (35) stored longleaf pine seeds in tight containers at low temperatures and found that they germinated well one or two years after collection. He suggested that this method would work equally well with seeds of slash, loblolly, and shortleaf pines.

Seeds of *Sequoia gigantea* were stored effectively though with decreasing viability for 18 years in an unsealed Mason jar at room temperature (Toumey 32). On the other hand, the same worker reported little or no germination of *Taxodium distichum* after dry storage for one winter.

Bates (5) stored white pine seeds for one year in containers sealed with paraffin. He found that seeds stored at low temperature, especially if they had been dried previously over H_2SO_4 (30 per cent relative humidity), gave a higher germination percentage in less time than did corresponding samples of fresh seed. The low moisture content was equally beneficial under all temperature conditions.

More recently Champion (8) reported that seeds of *Pinus longifolia* when sealed or mixed with charcoal in a gunny sack still gave good germination after two years. However, best results were obtained when the seeds were well dried and sealed before the damp season. High moisture content and variable temperatures were found by Coile (10) to cause a loss of vitality in slash pine seeds.

Isaac (21) demonstrated that not only did cold storage preserve Noble fir seed for five years without appreciable loss of vitality, but it actually seemed to increase the germinating power. The same author (22) showed

that Douglas fir seed if left on the forest floor either germinated or decayed within a year after it fell. Hence special storage is necessary. Baldwin (1) also reported the early loss of vitality of red spruce seeds when stored in duff, while during air-tight storage the germination decreased about 10 per cent per year for the first three years. Baldwin (2) further studied the effect of after-ripening treatment on the germination of white pine seeds of different ages and found no clear relation between the age of the seed and the effectiveness of after-ripening. The greatest effect was apparently produced in two- to three-year-old seed.

Guillaumin (19) studied the effect of an atmosphere devoid of oxygen as well as a vacuum on the keeping quality of grains. After six years he obtained no germination from those stored in air and 92 and 100 per cent respectively from those stored in an atmosphere devoid of O_2 and a vacuum.

Busse (7) has recently published a report of experiments in which he found a vacuum beneficial for the retention of vitality in pine and aspen seeds. He found the vacuum more effective in room temperature storage than in cellar storage and more beneficial for aspen than for pine seeds.

Smith (29) measured the CO_2 production in a dry pea seed and concluded that it would not exceed 1 cc. in three years at 12.5° C. This fact may be directly related to the beneficial effects of storage in a dry condition and at low temperatures.

Many other reports are available on the effects of temperature, sealing, and moisture content on the vitality of seeds of coniferous and other forms (12, 14, 15, 16, 17, 18, 23, 24, 25, 27, 28, 30, 34).

METHOD

In the storage experiments reported in this paper, the object was to determine the effects of sealing, temperature, vacuum, and a previous period of drying on the keeping quality of some coniferous forms. Sealing was in distillation flasks. In case the air was to be exhausted from the flask, the top was sealed after which the side tube was drawn out to a small bore and attached to an oil exhaust pump. When a good partial vacuum was obtained the side tube was sealed while still attached to the exhaust pump.

Drying in the first tests to be reported was accomplished by placing the seeds in desiccators over calcium oxide for 61 days previous to storage. In other tests to be described later varying amounts of quicklime were mixed with the seeds before sealing. Other methods used for *Pinus resinosa* Ait. will be described under the results of the tests for this form. Storage was at room temperature, 5° C., and in a refrigeration room with an average temperature of −15° C. for the first two or three years of the experiments, after which they were transferred to another refrigeration

room with an average temperature of $-5°$ C. Storage at room temperature was made in the dark as well as in the light. The "dark" flasks were put in a cardboard box and covered with black paper while the "light" flasks were put in an open basket in the laboratory. The flasks were all broken each time a vitality test was made, but they were restored to the original storage condition as soon as the samples were taken.

When vitality tests were made, one sample was always planted directly in soil in the greenhouse while another seed lot was mixed with moist granulated peat moss and placed at 5° C. Samples from this low temperature treatment were planted in the greenhouse after one or two, and in a few cases, after three months. Individual samples of 100 seeds each were used in most cases. In all cases, actual seedling production in soil was taken as an index of vitality.

It was very important, especially for some of the forms studied, to give the seeds a period at low temperature in a moist medium for after-ripening before planting in the greenhouse (3, 4). For example, in the tests of *Pinus taeda* L. if samples were planted directly in the greenhouse from the storage flask one would conclude that the vitality was lost after three years of storage, whereas, with a pre-treatment of one or two months in moist granulated peat moss at 5° C., a high percentage of sound seedlings was produced. This fact will be evident from the data presented below. *Pinus caribaea* and *Pinus ponderosa* showed the same marked effect of pre-treatment at low temperatures. To a less extent other forms studied responded in the same manner (Barton 4).

Here the importance of methods used in testing stored seeds becomes evident. Not only must a distinction be made between "germination" and "seedling production" (Haack 20), but the actual method employed must be described in order to make the results of vitality tests significant.

In order to conserve the supply of seeds in storage, no vitality tests were made in experiment 1 at the four-year-period. Tests were made after the seeds had been stored for one, two, three, five, six, and seven years.

In experiment 2, tests were made after one, two, four, five, and six years.

RESULTS AND DISCUSSION

LOBLOLLY PINE (PINUS TAEDA L.)

Experiment 1

The results of tests with these seeds are shown in Tables I and II and Figures 1 and 2.

Room temperature storage. When these seeds were stored in the dark unsealed they lost their vitality after one year. When air-dry seeds were sealed under the same conditions, the results were only slightly better.

Here there was a great decrease in vitality after one year and all of the seeds were dead after two years' storage.

TABLE I

SEEDLING PRODUCTION FROM "FRESH" SEEDS OF STORAGE EXPERIMENTS 1 AND 2

Species	Exp. No.	% seedling production after mos. at 5° C.		
		0	1	2
Pinus taeda	1 2	10 1	82 53	84 69
Pinus caribaea	1 2	10 31	74 58	78 66
Pinus echinata	1 2	20 42	59 42	84 82
Pinus palustris	1 2	74 46	77 61	— —
Pinus resinosa	1	25	—	—

However, when the flask was exhausted of air before storage, the seeds still held their vitality after seven years, although the power to produce seedlings had decreased by one-half in that time. It will be noted that low temperature pre-treatment seemed to be more essential to seedling production after three years of storage than before. This same fact will be seen in some of the other tests although in general the after-ripening effects remained constant throughout the duration of the various experiments.

Results from the unsealed lot at room temperature in the light showed the same effects as the dark storage. There was no unexhausted flask sealed in this case. However, there was a striking difference between the exhausted sealed condition here and in dark storage at the same temperature. In the light vitality was completely lost after two years. In view of other experiments to be described below this difference cannot be explained.

If the seeds were dried for 61 days over calcium oxide before storing in either light or dark at room temperature in a sealed flask with air, they did not remain viable for even one year. Hence, excessive drying was distinctly harmful under the conditions of this experiment. The effects of moderate drying were determined in another experiment reported below.

Storage at 5° C. This temperature proved very effective in maintaining seedling production ability of air-dry seeds practically unimpaired for seven years, whether the flasks were unsealed or sealed with or without air. If the seeds were dried before storage they maintained a fairly high vitality for two years when sealed in unexhausted flasks. The low temperature storage seemed to give some protection against injury from extreme

TABLE II

SEEDLING PRODUCTION FROM STORED SEEDS OF PINUS TAEDA. EXPERIMENT I

Storage condition			Per cent seedling production after storage for years																	
			1			2			3			5			6			7		
Relative moisture	Open or sealed	Temp.	G.H.* after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.		
			0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
Air-dry	Open	R.T.** dark	7	36	32	4	1	3	0	0	0	0	0	0	0	0	0	0	0	0
	Sealed	R.T. dark	13	82	78	23	24	25	0	2	1	0	0	0	0	0	0	0	0	0
	Vacuum	R.T. dark	15	83	87	58	55	76	1	51	67	7	73	59	2	60	60	19	45	45
	Open	R.T. light	2	40	32	2	9	5	—	—	—	—	—	—	—	—	—	—	—	—
	Vacuum	R.T. light	16	54	63	3	79	9	0	2	2	0	0	0	1	1	1	0	0	0
	Open	5° C.	11	86	87	64	82	89	8	77	81	13	64	74	2	64	64	28	60	66
	Sealed	5° C.	0	89	85	68	76	91	7	77	79	6	68	66	2	59	51	18	55	63
	Vacuum	5° C.	6	80	91	48	78	71	3	58	60	8	87	82	1	75	78	26	72	79
	Open	Refrig.***	1	76	89	56	71	85	9	81	83	5	87	89	2	80	86	42	89	83
	Sealed	Refrig.	1	74	79	58	76	73	10	61	72	11	62	77	3	73	71	29	80	73
	Vacuum	Refrig.	6	70	77	59	79	88	8	50	70	11	86	82	2	77	84	20	79	76
Dried over CaO	Sealed	R.T. dark	1	12	8	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sealed	R.T. light	1	6	8	0	0	0	1	0	0	0	0	0	0	0	0	—	—	0
	Sealed	5° C.	3	59	78	67	65	59	5	27	17	6	14	14	1	12	16	8	14	13
	Vacuum	5° C.	6	80	81	58	78	73	9	43	64	11	55	66	0	57	62	27	—	69
	Sealed	Refrig.	2	79	78	71	75	80	5	63	81	14	69	84	—	—	—	34	91	85
	Vacuum	Refrig.	3	73	82	59	67	70	7	42	65	6	69	81	—	—	—	35	70	75

* Greenhouse; ** Room temperature; *** Refrigeration room.

drying. After this time, however, the decline in germinative power was rapid, the seedling production being only 27 per cent after three years. If the seeds were dried, the vacuum storage seemed to favor longevity, especially for the longer storage periods. Seeds from unexhausted and exhausted flasks still gave about the same percentage of seedling production after two years' storage. After three years, however, the seedling production was 17 and 64 per cent respectively; after five years, 14 and 66 per

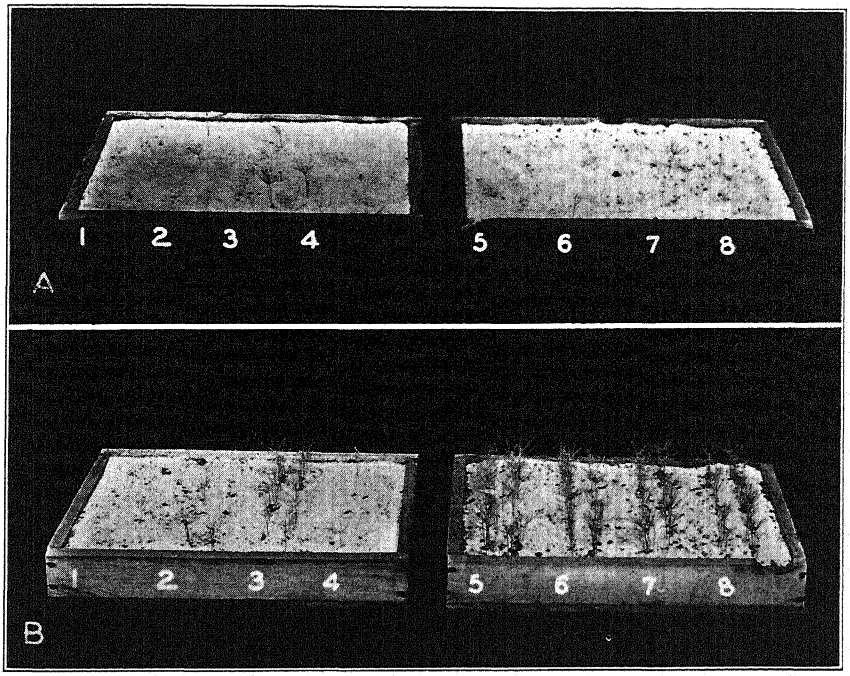

FIGURE 1. *Pinus taeda.* Seedling production in the greenhouse after storage for one year (air-dry seeds). A. Planted directly in flat from storage flasks. (1) Open room temperature dark, (2) sealed room temperature dark, (3) vacuum room temperature dark, (4) open room temperature light, (5) vacuum room temperature light, (6) open 5° C., (7) sealed 5° C., (8) vacuum 5° C. B. Planted in flat after one month in moist granulated peat moss at 5° C. Numbers as in A.

cent; after six years, 16 and 62 per cent; and after seven years, 13 and 69 per cent. These results followed pre-treatment for two months at 5° C. in each case (Table II).

Refrigeration room storage (−15° *and* −5° *C.*). Air-dry seeds at this temperature gave results similar to those stored at 5° C. However, dried seeds differed in that excellent seedling production was obtained from both unexhausted and exhausted sealed conditions after seven years. The

below-freezing temperatures apparently made the vacuum unnecessary or ineffective, and overcame the injurious effects of extreme pre-drying.

Experiment 2

A second similar storage experiment of seeds of *P. taeda* was started a year later (1929). The results are shown in Table III.

Room temperature storage. Results from the room temperature storage, light and dark, were the same as for the previous experiment except that

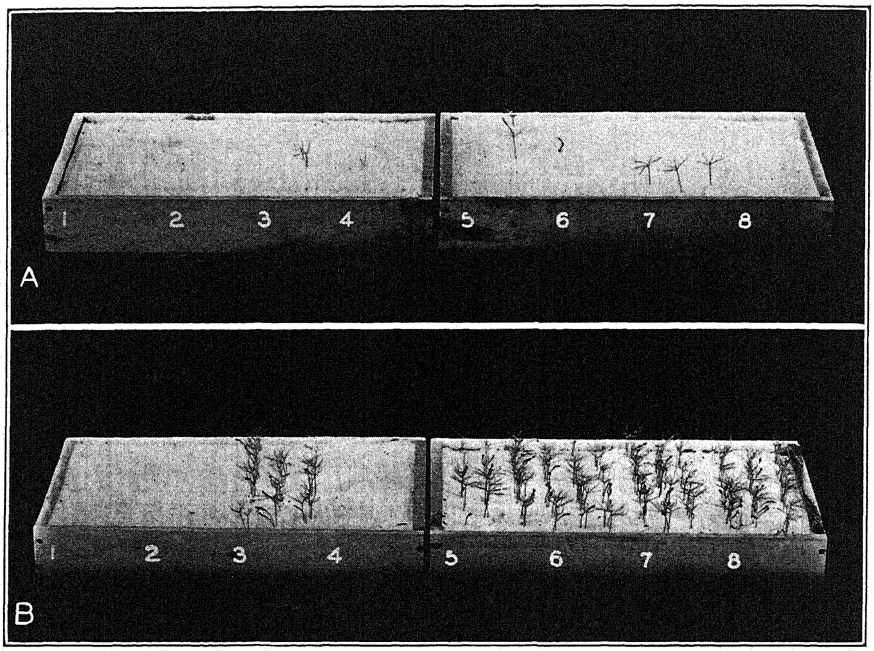

FIGURE 2. *Pinus taeda.* Seedling production in the greenhouse after storage for five years (air-dry seeds). A. Planted directly in flat from storage flasks. (1) Open room temperature dark, (2) sealed room temperature dark, (3) vacuum room temperature dark, (4) vacuum room temperature light, (5) open 5° C., (6) sealed 5° C., (7) vacuum 5° C., (8) open refrigeration room. B. Planted in flat after one month in moist granulated peat moss at 5° C. Numbers as in A.

the vacuum effects were reversed. In other words, seeds from the exhausted flasks stored in the light still retained their vitality after five years while those in the dark showed no such beneficial effects.

In this experiment dried seeds were stored in vacuum as well as in air at room temperature. The results confirmed those obtained for 5° C. in the previous experiment in that a vacuum was necessary to maintain the vitality of dried seeds at temperatures above freezing. One might

TABLE III

SEEDLING PRODUCTION FROM STORED SEEDS OF PINUS TAEDA. EXPERIMENT 2

Storage condition			Per cent seedling production after storage for years													
			1		2			4			5			6		
Relative moisture	Open or sealed	Temp.	G.H. after mos. at 5° C.		G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.		
			0	1	0	1	2	0	1	2	0	1	2	0	1	2
Air-dry	Vacuum	R.T. dark	0	1	0	0	2	0	0	0	0	0	0	0	0	0
	Sealed	R.T. dark	0	2	0	0	13	0	0	0	0	0	0	0	0	1
	Open	R.T. dark	1	13	0	7	4	0	0	0	0	0	0	0	0	0
	Vacuum	R.T. light	3	17	29	17	39	6	19	35	0	5	40	0	15	26
	Sealed	R.T. light	0	13	0	5	0	0	0	0	0	0	0	0	0	0
	Open	R.T. light	1	14	11	11	22	0	0	2	0	0	0	0	0	0
	Vacuum	5° C.	1	41	16	31	59	2	38	49	1	24	37	—	43	44
	Sealed	5° C.	1	36	15	19	46	7	36	56	0	12	48	1	52	43
	Open	5° C.	1	86	4	17	46	3	32	46	0	17	50	1	40	52
	Vacuum	Refrig.	4	24	3	29	42	3	41	56	1	32	54	3	43	47
	Sealed	Refrig.	2	23	3	24	42	2	26	58	0	21	77	7	59	56
	Open	Refrig.	2	10	3	31	55	2	28	50	0	17	68	6	49	62
Dried over CaO	Vacuum	R.T. dark	7	35	8	38	47	2	35	46	2	34	57	2	—	40
	Sealed	R.T. dark	2	15	6	0	2	0	0	0	0	0	0	—	—	—
	Vacuum	R.T. light	3	40	6	48	46	2	40	52	3	16	32	1	—	11
	Sealed	R.T. light	—	—	4	12	15	0	5	3	0	3	1	0	0	1
	Vacuum	5° C.	0	37	8	38	52	6	51	51	1	32	53	0	—	—
	Sealed	5° C.	3	42	4	32	42	1	36	42	0	23	56	0	—	44
	Vacuum	Refrig.	3	21	0	20	33	3	44	46	0	26	45	0	—	—
	Sealed	Refrig.	2	31	0	16	40	4	41	51	1	19	72	0	46	56

expect the differences to be more marked at higher storage temperatures and such is the case. Whereas seeds from unexhausted sealed flasks were practically all dead after two years of storage, those from vacuum were still giving excellent seedling production after five years.

Storage at 5° C. Results here were practically the same as in experiment 1. There was not such a conspicuous difference in the seedling production from dried seeds stored in unexhausted and exhausted flasks as in the previous case, but a small difference was apparent.

Refrigeration room storage. These tests corroborated the findings in the first experiment.

SLASH PINE (PINUS CARIBAEA MORELET)

Experiment 1

Room temperature storage. Dark. Unsealed seeds lost their vitality after two years of storage; the seedling production was practically nil after three years. In sealed storage, the vitality was very much decreased after two years but there was still some seedling production after six years. Vacuum had an effect similar to ordinary sealed storage.

Light. No seeds were available for tests of the unsealed lot after the two year period at which time many of the seeds were still viable. Both these seeds and those from unexhausted sealed flasks were similar in their behavior to those from dark storage. The vacuum again (as noted in *P. taeda*, experiment 2) was very effective in keeping the quality of the seeds. Fairly good seedling production was obtained after seven years although a slight gradual decrease was noted (Table IV).

Dried seeds in unexhausted sealed flasks were dead before the end of the first year's storage (cf. *P. taeda*, experiments 1 and 2).

Storage at 5° C. All air-dry seeds retained germinative power for five years. After seven years, however, the seeds from the open container showed a greater decrease in vitality than those stored in sealed containers.

Dried seeds at this temperature whether sealed with air or in vacuum kept their vitality for two years. No tests were made after three or four years, but a test after five years showed a decided decline in germinative ability with the vacuum again showing a slight superiority (Table IV. cf. *P. taeda*). Dried seeds were inferior in keeping quality to air-dry seeds,

Refrigeration room storage. This temperature had the same effects on slash pine seeds as 5° C. There is some indication that this colder storage temperature was better than 5° C. for overcoming the unfavorable effect of excess drying when the storage flasks were not evacuated. No seeds were available for tests for periods longer than five years.

Experiment 2

Room temperature storage. As in experiment 1, open flasks in both light and dark maintained the germination power of these seeds for two years

TABLE IV

SEEDLING PRODUCTION FROM STORED SEEDS OF PINUS CARIBAEA. EXPERIMENT I

Storage condition			Per cent seedling production after storage for years																	
			1			2			3			5			6			7		
Relative moisture	Open or sealed	Temp.	G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.		
			0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
Air-dry	Open	R.T. dark	12	52	53	39	41	36	4	4	4	0	0	0	0	0	0	—	—	—
	Sealed	R.T. dark	11	67	73	43	41	56	5	33	27	9	12	18	1	6	9	—	—	—
	Vacuum	R.T. dark	14	76	84	54	30	42	11	17	15	7	16	8	3	12	15	—	—	—
	Open	R.T. light	3	25	29	28	18	17	—	—	—	—	—	—	—	—	—	—	—	—
	Vacuum	R.T. light	14	66	77	33	69	61	15	42	59	11	60	50	—	—	—	—	—	—
	Open	5° C.	18	72	81	40	64	74	1	55	75	4	60	61	—	—	—	—	—	44
	Sealed	5° C.	27	82	89	66	88	80	17	65	59	21	71	81	—	—	—	—	—	49
	Vacuum	5° C.	20	77	73	55	73	79	13	72	88	19	76	79	7	65	79	—	—	78
	Open	Refrig.	10	61	75	46	85	80	16	63	80	12	61	81	—	—	—	36	64	68
	Sealed	Refrig.	15	68	74	52	82	85	19	68	83	26	62	85	—	—	—	—	—	—
	Vacuum	Refrig.	18	72	65	54	78	72	20	65	75	23	55	61	—	—	—	44	72	65
Dried over CaO	Sealed	R.T. dark	4	11	6	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—
	Sealed	R.T. light	6	15	14	0	1	1	2	0	0	0	0	—	—	—	—	—	—	—
	Sealed	5° C.	8	50	66	40	58	58	—	—	—	3	20	30	—	—	—	—	—	—
	Vacuum	5° C.	27	62	60	74	70	77	—	—	—	20	35	—	—	—	—	—	—	—
	Sealed	Refrig.	7	58	61	40	62	57	—	—	—	9	46	—	—	—	—	—	—	—
	Vacuum	Refrig.	24	67	80	44	51	67	—	—	—	11	31	—	—	—	—	—	—	—

TABLE V

SEEDLING PRODUCTION FROM STORED SEEDS OF PINUS CARIBAEA. EXPERIMENT 2

Storage condition			Per cent seedling production after storage for years														
			1		2			4			5			6		
			G.H. after mos. at 5° C.		G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.		
Relative moisture	Open or sealed	Temp.	0	1	0	1	2	0	1	2	0	1	2	0	1	2
Air-dry	Vacuum	R.T. dark	8	46	13	36	25	4	2	1	0	0	0	0	0	0
	Sealed	R.T. dark	10	34	23	36	45	18	28	29	2	27	26	7	—	25
	Open	R.T. dark	16	53	9	27	31	1	0	0	0	0	0	3	—	0
	Vacuum	R.T. light	14	47	21	45	40	13	49	45	8	57	52	23	50	41
	Sealed	R.T. light	21	67	21	36	41	17	34	36	6	30	41	14	22	12
	Open	R.T. light	8	58	15	37	43	1	0	1	0	0	0	1	0	0
	Vacuum	5° C.	29	57	38	56	69	20	54	62	10	50	45	24	68	57
	Sealed	5° C.	26	44	11	36	41	20	63	70	9	57	57	25	53	55
	Open	5° C.	21	50	23	75	52	18	58	47	9	41	45	11	56	44
	Vacuum	Refrig.	32	55	16	72	100	16	70	68	13	65	55	38	67	61
	Sealed	Refrig.	20	47	16	47	27	13	65	63	12	64	68	21	66	56
	Open	Refrig.	24	73	13	63	57	25	59	57	3	61	68	23	70	56
Dried over CaO	Vacuum	R.T. dark	4	8	—	—	—	6	2	—	0	1	—	—	—	—
	Sealed	R.T. dark	0	0	0	0	2	0	0	0	0	—	—	—	—	—
	Vacuum	R.T. light	2	0	—	—	—	2	1	0	0	0	—	—	—	—
	Sealed	R.T. light	0	0	0	0	21	0	0	0	—	—	—	—	—	—
	Vacuum	5° C.	14	40	—	—	—	18	59	—	5	33	—	—	—	—
	Sealed	5° C.	—	—	4	26	0	8	7	—	0	8	0	—	—	—
	Vacuum	Refrig.	15	52	—	—	—	21	27	54	4	47	—	—	—	—
	Sealed	Refrig.	10	24	—	—	—	13	47	38	—	—	—	—	—	—

TABLE VI

SEEDLING PRODUCTION FROM STORED SEEDS OF PINUS ECHINATA. EXPERIMENT 1

Storage condition			Per cent seedling production after storage for years																	
			1			2			3			5			6			7		
Relative moisture	Open or sealed	Temp.	G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.		
			0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
Air-dry	Open	R.T. dark	2	52	54	16	16	19	1	4	6	1	0	0	0	0	0	0	0	0
	Sealed	R.T. dark	12	60	67	41	40	43	6	16	12	3	13	14	1	5	6	4	2	3
	Vacuum	R.T. dark	11	64	52	35	36	28	9	7	8	3	19	9	5	10	7	3	6	3
	Open	R.T. light	1	14	25	17	8	8	—	—	—	—	—	—	—	—	—	—	—	—
	Vacuum	R.T. light	9	41	40	36	53	60	14	32	31	20	43	30	14	43	27	21	37	28
	Open	5° C.	6	64	62	71	67	70	22	73	56	16	49	49	14	64	43	32	51	50
	Sealed	5° C.	5	61	63	45	77	66	9	19	16	30	22	27	13	43	35	17	17	21
	Vacuum	5° C.	27	79	87	73	57	53	17	29	39	49	62	62	26	77	60	32	36	32
	Open	Refrig.	10	43	32	65	70	62	45	73	34	42	72	78	21	63	67	64	69	76
	Sealed	Refrig.	14	63	58	28	43	46	8	48	24	22	49	40	27	52	41	15	38	29
	Vacuum	Refrig.	12	57	54	78	87	88	23	60	61	38	74	62	47	76	74	72	93	89
Dried over CaO	Sealed	R.T. dark	1	21	14	8	1	6	1	0	0	0	0	0	0	0	0	0	0	0
	Sealed	R.T. light	1	9	18	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	Sealed	5° C.	5	45	60	37	40	34	12	15	1	26	12	12	16	43	40	22	37	30
	Vacuum	5° C.	4	42	41	57	59	54	26	49	27	29	22	19	38	49	45	31	48	45
	Sealed	Refrig.	17	81	76	64	65	58	38	48	33	56	61	58	37	61	60	34	52	51
	Vacuum	Refrig.	7	31	56	59	48	52	11	54	56	26	40	30	50	76	59	58	57	56

only (Table V). Each experiment showed a sharp drop in seedling production from fairly good after two years to practically zero after three years of storage. Drying the seeds as in the previous experiment was decidedly harmful. Only occasional seedlings were ever obtained from these seeds.

Storage at 5° C. Results here were the same as for the preceding experiment. The vacuum again proved best for storing dried seeds at this temperature, giving 33 per cent seedling production after five years as against 8 per cent from an unexhausted flask (Table V).

Refrigeration room storage. Results were similar to those in experiment 1.

SHORTLEAF PINE (PINUS ECHINATA MILL.)

Experiment 1

Room temperature storage. Dark. Unsealed seeds showed a decrease in vitality after one year of storage (Table VI). Seedling production was very much reduced after three years' storage and was nil after five years. Sealed storage whether exhausted or unexhausted was only slightly better.

Light. Unsealed storage did not keep the seeds well for even one year and seedling production was very poor after two years. Exhausted sealed flasks kept some of the seeds viable for seven years.

Dried seeds in unexhausted sealed flasks in both light and dark lost practically all of their vitality during the first year. There were practically no seedlings from seeds stored two years.

Storage at 5° C. This temperature was effective whether the seeds were unsealed, or sealed with or without air. A steady decline brought the seedling production to about one-third of the original after seven years of storage.

Dried seeds germinated after seven years in unexhausted or exhausted sealed flasks with the former lagging behind the latter in germination capacity.

Refrigeration room storage. Storage at this temperature had the same effects on the keeping quality of shortleaf pine as already described for loblolly and slash pines.

Experiment 2

Results were the same in general as for experiment 1 (Table VII).

LONGLEAF PINE (PINUS PALUSTRIS MILL.)

Experiment 1

Seeds available for this experiment permitted vitality tests only after one and two years. The results are shown in Table VIII.

Room temperature storage. Both light and dark storage at this temperature were ineffectual. Practically all vitality was lost in one year if the

TABLE VII

SEEDLING PRODUCTION FROM STORED SEEDS OF PINUS ECHINATA. EXPERIMENT 2

Storage condition			Per cent seedling production after storage for years													
			1		2			4			5			6		
Relative moisture	Open or sealed	Temp.	G.H. after mos. at 5° C.		G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.		
			0	1	0	1	2	0	1	2	0	1	2	0	1	2
Air-dry	Vacuum	R.T. dark	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sealed	R.T. dark	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	Open	R.T. dark	33	51	7	27	12	0	0	0	0	0	0	0	0	0
	Vacuum	R.T. light	5	2	2	0	5	0	0	0	0	0	0	0	0	0
	Sealed	R.T. light	13	26	2	5	5	0	0	0	0	0	0	0	0	0
	Open	R.T. light	25	59	31	51	43	0	0	2	0	1	0	0	0	0
	Vacuum	5° C.	36	65	47	61	72	57	74	64	13	71	64	58	79	70
	Sealed	5° C.	52	62	37	67	71	51	63	74	17	79	88	55	77	65
	Open	5° C.	37	54	50	53	53	36	53	61	10	59	58	32	51	47
	Vacuum	Refrig.	51	71	55	56	56	50	56	73	30	73	86	61	65	66
	Sealed	Refrig.	28	63	46	52	59	33	59	65	21	65	78	49	66	50
	Open	Refrig.	46	60	51	64	36	56	74	76	11	70	60	63	71	73
Dried over CaO	Vacuum	R.T. dark	43	69	—	—	—	49	64	49	28	56	57	46	71	49
	Sealed	R.T. dark	36	24	4	13	14	0	0	0	0	0	1	1	0	0
	Vacuum	R.T. light	40	55	—	—	—	0	0	0	0	0	0	0	1	0
	Sealed	R.T. light	30	38	41	46	37	32	46	50	10	40	34	7	16	9
	Vacuum	5° C.	28	48	40	50	63	42	54	53	20	49	61	51	57	49
	Sealed	5° C.	34	66	47	76	70	23	54	59	14	52	59	45	74	67
	Vacuum	Refrig.	39	50	—	—	—	53	60	74	31	69	74	51	67	68
	Sealed	Refrig.	35	64	38	78	66	42	78	67	34	71	84	53	84	70

seeds were stored air-dry and no seedlings were ever obtained from the dried seeds.

Storage at 5° C. At this temperature there was good retention of vitality for two years.

Refrigeration room storage. Results were the same as for 5° C. Exhausted flasks in which dried seeds were stored proved better than unexhausted flasks for retaining vitality.

TABLE VIII

SEEDLING PRODUCTION FROM STORED SEEDS OF PINUS PALUSTRIS. EXPERIMENT 1

Storage condition			Per cent seedling production after storage for years					
			1			2		
Relative moisture	Open or or sealed	Temp.	G.H. after mos. at 5° C.			G.H. after mos. at 5° C.		
			0	1	2	0	1	2
Air-dry	Open	R.T. dark	0	10	6	—	—	—
	Sealed	R.T. dark	1	0	3	3	0	0
	Vacuum	R.T. dark	3	19	9	2	6	18
	Open	R.T. light	0	5	4	0	0	3
	Vacuum	R.T. light	2	10	7	3	0	0
	Open	5° C.	41	71	70	40	60	38
	Sealed	5° C.	38	68	69	53	73	75
	Vacuum	5° C.	49	66	65	55	56	27
	Open	Refrig.	57	68	70	72	86	73
	Sealed	Refrig.	56	79	85	40	75	63
	Vacuum	Refrig.	51	86	54	34	66	52
Dried over CaO	Sealed	R.T. dark	0	0	0	0	0	0
	Sealed	R.T. light	0	0	0	0	0	0
	Sealed	5° C.	4	25	29	—	—	—
	Vacuum	5° C.	11	40	48	47	20	38
	Sealed	Refrig.	6	40	33	30	—	—
	Vacuum	Refrig.	7	52	47	32	28	42

Experiment 2

Tests in this case were made after one, two, four, five, and six years (Table IX).

Room temperature storage. Results similar to those for experiment 1 were obtained.

Storage at 5° C. There was fairly good seedling production after two years in all conditions, but after four years of storage, results from seeds from exhausted flasks were twice as good as those from unexhausted sealed flasks and 16 times as good as those unsealed. However, the vitality had decreased tremendously at this time. These seeds were injured by drying so that very poor germination was ever obtained from this temperature.

Refrigeration room storage. This temperature proved far superior to the other two used in maintaining the vitality of longleaf pine seeds. Even after five years of storage, about 0.8 of original vitality remained if the seeds were given a pre-treatment of cold and about 0.5 when the seeds were planted directly in greenhouse flats.

In spite of the favorable temperature, however, the harmful effect of drying was still evident.

TABLE IX

SEEDLING PRODUCTION FROM STORED SEEDS OF PINUS PALUSTRIS. EXPERIMENT 2

Storage condition			Per cent seedling production after storage for years												
			1		2			4			5			6	
Relative moisture	Open or sealed	Temp.	G.H. after mos. at 5° C.		G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.	
			0	1	0	1	2	0	1	2	0	1	2	0	1
Air-dry	Vacuum	R.T. dark	0	0	0	0	0	0	0	—	0	0	—	0	—
	Sealed	R.T. dark	0	0	0	0	0	0	0	—	0	0	—	0	—
	Open	R.T. dark	2	18	2	0	0	0	0	—	0	0	—	0	—
	Vacuum	R.T. light	0	0	0	0	0	0	0	—	0	0	—	0	—
	Sealed	R.T. light	0	0	0	0	0	0	0	—	0	0	—	0	0
	Open	R.T. light	5	10	0	4	0	0	0	—	0	0	—	—	—
	Vacuum	5° C.	24	42	38	42	27	16	16	—	6	30	—	17	17
	Sealed	5° C.	15	26	22	20	13	12	8	—	1	1	—	5	—
	Open	5° C.	18	36	20	32	9	1	1	—	0	0	—	0	—
	Vacuum	Refrig.	31	48	40	48	25	30	43	—	22	50	—	37	—
	Sealed	Refrig.	31	38	32	52	34	29	35	—	15	36	—	21	—
	Open	Refrig.	31	40	46	58	21	18	33	—	5	28	—	—	—
Dried over CaO	Vacuum	R.T. dark	14	24	—	—	—	13	6	—	0	18	—	—	—
	Sealed	R.T. dark	0	0	0	0	2	0	0	—	0	—	—	—	—
	Vacuum	R.T. light	2	0	—	—	—	0	0	—	0	0	—	—	—
	Sealed	R.T. light	5	22	0	8	5	0	0	—	—	—	—	—	—
	Vacuum	5° C.	4	10	—	—	—	6	10	—	3	6	—	4	—
	Sealed	5° C.	22	48	—	—	—	5	19	—	—	—	—	—	—
	Vacuum	Refrig.	13	34	—	—	—	11	8	—	4	14	—	—	—
	Sealed	Refrig.	18	36	—	—	—	16	37	—	4	—	—	—	—

RED PINE (PINUS RESINOSA AIT.)

Tests with these seeds differed from others described above in that additional methods of drying were used. Samples were placed in an oven at 51° to 53° C. for 24 hours before sealing. Other lots were dried for eight weeks over calcium chloride, lithium chloride, or potassium acetate.

Room temperature storage. Unsealed lots of air-dry seeds in the dark at this temperature lost their vitality almost completely before the end of three years of storage. Sealed dark storage in vacuum permitted the production of only a few seedlings after seven years of storage while light storage in vacuum was much more effective (Table X).

TABLE X

SEEDLING PRODUCTION FROM STORED SEEDS OF PINUS RESINOSA. EXPERIMENT 1

Storage condition			Per cent seedling production after storage for years																	
			1			2			3			5			6			7		
Relative moisture	Open or sealed	Temp.	G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5° C.			G.H. after mos. at 5 °C.		
			0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
Air-dry	Open	R.T. dark	18	39	58	33	54	29	1	3	0	0	0	1	0	0	0	—	—	—
	Vacuum	R.T. dark	42	68	68	46	60	46	23	29	5	12	13	6	2	10	5	6	12	2
	Vacuum	R.T. light	42	43	67	46	56	46	23	39	4	31	42	37	25	56	39	43	43	34
	Open	5° C.	32	44	53	30	51	41	33	31	19	40	44	32	46	57	30	37	26	17
	Vacuum	5° C.	39	54	63	40	52	54	22	38	27	38	45	43	42	50	31	47	48	34
	Open	Refrig.	25	53	56	28	49	44	24	32	40	42	55	51	27	61	38	38	48	37
	Vacuum	Refrig.	37	42	63	28	57	44	39	38	25	30	54	31	40	58	—	40	54	35
Dried over CaO	Sealed	R.T. dark	0	1	0	0	0	2	0	0	0	0	0	0	0	—	—	—	—	—
	Sealed	R.T. light	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—
	Sealed	5° C.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Vacuum	5° C.	0	0	0	1	—	0	0	0	0	0	0	0	0	—	—	—	—	—
Dried in oven 51° to 53° C.	Sealed	R.T. dark	32	35	45	22	24	19	—	—	—	—	—	—	—	—	—	—	—	—
	Sealed	R.T. light	29	46	38	19	14	21	—	—	—	—	—	—	—	—	—	—	—	—
	Vacuum	5° C.	48	59	64	48	50	65	—	—	—	—	—	—	—	—	—	—	—	—
	Sealed	Refrig.	41	40	62	50	42	47	—	—	—	—	—	—	—	—	—	—	—	—
Dried over calcium chloride	Sealed	R.T. dark	35	55	52	8	6	6	1	4	1	1	0	1	0	2	0	0	—	0
	Sealed	R.T. light	32	63	53	15	26	15	4	2	0	0	0	0	0	0	—	—	—	—
	Sealed	5° C.	35	66	51	48	59	48	30	37	54	45	62	40	38	51	39	43	50	26
	Vacuum	Refrig.	43	58	51	51	58	40	6	34	10	37	42	34	—	—	—	47	44	32
Dried over $KC_2H_3O_2$	Sealed	R.T. dark	28	44	38	12	4	7	0	0	0	0	0	0	0	0	0	0	—	0
	Sealed	R.T. light	19	49	42	4	9	8	1	0	0	1	1	0	0	0	0	—	0	0
	Sealed	5° C.	50	57	68	48	51	43	5	18	15	36	44	44	33	54	42	21	—	33
	Vacuum	Refrig.	51	67	70	61	64	51	7	23	4	46	65	55	38	60	51	42	52	41
Dried over lithium chloride	Sealed	R.T. dark	28	58	52	23	29	20	5	20	0	0	0	0	1	0	0	0	—	0
	Sealed	R.T. light	37	54	55	13	18	26	10	5	1	1	1	0	0	0	0	0	0	0
	Sealed	Refrig.	40	58	43	62	50	51	11	21	28	48	55	53	—	—	—	40	44	41
	Vacuum	Refrig.	41	33	53	59	55	41	22	16	9	27	53	50	36	64	—	39	45	41

When seeds were dried over CaO and stored sealed at room temperature they were dead before the end of the first year. Seeds otherwise dried (described above) were not so seriously injured although in all cases there was very poor seedling production after two years of storage.

Storage at 5° C. After seven years of storage at this temperature especially if the storage flasks were evacuated the vitality seemed unimpaired.

Again the period of drying over CaO proved detrimental to the vitality of these seeds. Other drying methods used did not seem to harm the seeds if they were stored at this low temperature.

Refrigeration room storage. Results were practically the same as for 5° C. storage.

EFFECT OF CALCIUM OXIDE WHEN MIXED WITH STORED SEEDS

Seeds of *Pinus palustris*, *Pinus taeda*, *Pinus ponderosa* Douglas, *Picea abies* (L.) Karst. (*P. excelsa* Link), and *Picea canadensis* (Mill.) BSP. of this storage lot were mixed directly with CaO to obtain drying effects. Sealing was effected here by using bottles fitted with tight stoppers and sealed with Dekhotinsky cement. Moisture determinations were made at the beginning of the experiment for the pines studied and for all seeds each time a germination test was made thereafter. These tests were made by drying in a 60° C. vacuum oven to a constant weight. On the basis of the original moisture content of *Pinus palustris* and *Pinus taeda* seeds, they were mixed with different quantities of calcium oxide to remove approximately 0.3, 0.5, 0.8, and all of the moisture present at the time of storage. Two lots with surplus amounts were also stored (3.11 g. CaO removes 1 g. water).

In the case of the other three species where the available quantity of seeds was more limited, seeds were stored with amounts of quicklime sufficient to remove approximately one-third, two-thirds, and all of the moisture. Original moisture content and seedling production of these seeds are given in Table XI. The weights of the seeds stored together with the weights of CaO are shown in Table XII. Since a fairly large amount of seeds was stored in each bottle, two or more tests were made from each. This means that a bottle may have been opened at the end of a year and a sample removed after which the bottle with the original amount of calcium oxide was resealed. However, this opening of the bottles did not seem to have influenced subsequent storage effects. Seeds from bottles opened for the first time after four years of storage had same germination capacity as those which had been opened one or more times. Control lots of seeds were sealed without calcium oxide and in the case of *Pinus ponderosa* one lot was stored open. Storage was in a cold room with an average of about −15° C. from the beginning until October 1933, at which time they were transferred to a cold room of about −5° C. Seeds of *Pinus*

palustris and *Pinus taeda* were stored July 25, 1928, those of *Pinus ponderosa* February 2, 1929, and those of *Picea excelsa* and *Picea canadensis* on November 14, 1928. Subsequent vitality tests were made in the manner described above and at the same time for all species.

Moisture Tests

As will be seen from Tables XI and XII moisture contents after storage varied from year to year, but with a few exceptions they remained in the same order for each test. In other words, seeds which were air-dried nearly always had the highest moisture content while those mixed with the greatest amounts of quicklime had the lowest moisture contents. There was a general tendency for the moisture content to become fixed after two years of storage. This tendency became even more marked after four years of storage. The failure to obtain consistent results from these moisture determinations might be due to the error introduced by opening the bottles for samples or to the time required by the seeds in storage to bring their moisture content to an equilibrium. More likely, however, it is due to the inefficiency of the moisture determination method although every effort was made to keep the conditions uniform from year to year. There is great need for a standardization of methods for determining the moisture content of widely different types of seeds.

TABLE XI

ORIGINAL MOISTURE AND SEEDLING PRODUCTION TESTS OF VARIOUS SEEDS MIXED WITH DIFFERENT AMOUNTS OF CaO

Species	Per cent moisture	Per cent seedling production after mos. at 5° C.		
		0	1	2
Pinus palustris	9.8	5	17*	63*
Pinus taeda	11.4	2	42	52
Pinus ponderosa	3.7	50	38	47
Picea excelsa	—	53	65	57
Picea canadensis	—	48	48	16

* Pre-treated at 1° C. instead of 5° C.

Vitality Tests

Pinus palustris. Practically all of the moisture can be removed from these seeds without killing the seeds, although seedling production was reduced after storage for one year with amounts of CaO sufficient to remove all of the water or with even greater amounts. The same proportional decrease in germination ability with excessive drying is evident after one, two, four, and five years of storage (Table XII).

TABLE XII

VITALITY TESTS OF VARIOUS SEEDS MIXED WITH DIFFERENT AMOUNTS OF CaO AND STORED SEALED IN REFRIGERATION ROOM

Species	Storage	Stored 1 yr.				Stored 2 yrs.				Stored 4 yrs.				Stored 5 yrs.				Stored 6 yrs.			
	Seeds +CaO (grams)	% moisture	% seedling production G.H. after mos. at 5° C.			% moisture	% seedling production G.H. after mos. at 5° C.			% moisture	% seedling production G.H. after mos. at 5° C.			% moisture	% seedling production G.H. after mos. at 5° C.			% moisture	% seedling production G.H. after mos. at 5° C.		
			0	1	2		0	1	2		0	1	2		0	1	2		0	1	2
Pinus palustris	100+ 0	5.5	22	20	49	8.2	10	44	80	10.1	10	14	—	—	—	—	—	—	—	—	—
	100+10	2.6	12	20	53	5.9	18	44	72	7.5	15	11	—	5.9	4	12	50	5.9	14	23	—
	100+17	1.2	5	21	45	4.4	12	20	64	5.7	17	5	3	4.7	12	9	36	4.8	5	40	—
	100+25	0.4	11	8	40	2.6	10	16	48	3.8	17	13	—	3.4	11	—	35	3.3	17	7	—
	100+32	4.0	7	10	39	2.2	12	8	48	2.1	6	4	—	2.2	9	3	16	1.9	7	22	—
	100+45	0.0	6	8	16	2.3	7	14	37	2.5	9	7	20	1.5	2	3	10	0.0	3	8	—
	100+64	0.0	6	12	33	1.9	6	12	57	2.0	6	5	13	1.0	1	3	12	0.9	2	6	—
Pinus taeda	100+ 0	6.1	5	40	68	10.1	2	32	45	11.3	1	46	63	10.5	2	50	64	10.8	1	54	70
	100+10	3.5	2	56	68	7.1	1	29	68	8.4	2	60	59	7.9	3	40	61	8.5	4	54	71
	100+17	1.8	2	39	63	5.1	3	24	60	6.2	2	53	69	5.9	3	34	48	6.2	1	49	73
	100+25	0.5	3	43	54	—	1	37	51	5.2	0	52	53	4.9	10	44	50	5.3	2	47	49
	100+32	—	2	45	55	2.3	8	39	61	2.7	1	55	57	2.5	3	51	58	2.9	1	47	55
	100+45	0.0	3	51	50	1.6	5	31	58	1.6	1	61	50	0.9	—	34	43	0.7	0	37	44
	50+32	0.0	9	48	61	1.1	4	21	65	1.2	2	47	57	0.6	—	13	26	0.7	0	—	24
Pinus ponderosa	50+ 0	0.8	40	66	63	4.5	8	64	60	6.2	9	50	58	—	—	—	—	—	—	—	—
	50+ 2	0.3	21	78	50	4.1	24	70	65	6.1	22	31	54	—	—	—	—	—	—	—	—
	50+ 4	0.04	28	68	59	3.3	12	62	67	5.5	33	54	60	—	—	—	—	—	—	—	—
	50+ 6	0.0	15	70	69	2.8	28	62	60	2.5	24	58	66	—	—	—	—	—	—	—	—
	50+ 0*	1.0	20	62	65	5.2	18	36	73	10.2	16	55	60	—	—	—	—	—	—	—	—
Picea excelsa	50+ 0	1.4	50	26	36	4.6	57	36	39	6.4	48	53	49	5.8	50	49	33	0.0	56	59	49
	50+ 8	0.1	63	35	40	2.0	58	40	37	2.5	50	53	40	1.8	40	33	22	1.6	42	38	23
	50+15	0.0	54	39	—	1.6	64	38	19	2.0	42	35	18	0.9	20	24	7	0.6	8	20	4
	50+30	0.0	57	28	40	1.4	61	34	39	1.2	27	34	13	0.4	9	5	2	0.3	7	3	3
Picea canadensis	25+ 0	1.1	55	54	58	4.2	56	77	64	5.6	53	73	61	5.2	58	91	69	5.8	57	78	72
	25+ 4	0.4	64	56	60	2.6	54	73	87	2.5	54	79	68	2.0	35	83	76	1.7	32	81	77
	25+ 8	0.0	61	79	65	2.1	63	73	80	1.7	33	84	66	0.9	14	72	35	0.9	14	60	57
	25+15	0.2	63	71	71	1.8	51	78	82	1.3	31	73	45	0.8	21	88	45	0.5	9	53	59

* Open.

While fresh longleaf pine seeds do not require previous low temperature treatment to obtain good seedling production in the greenhouse [the only effect of such treatment being to hasten the germination (3)], as the vitality falls in storage, a period of two months at 5° C. previous to planting in a greenhouse flat becomes highly effective in bringing about seedling production. This is true both in the more and less favorable storage conditions. For instance, after two years of storage with one-third of moisture removed seeds planted in the greenhouse immediately gave 18 per cent seedling production while those pre-treated in moist granulated peat moss for two months prior to planting gave 72 per cent. Compa-

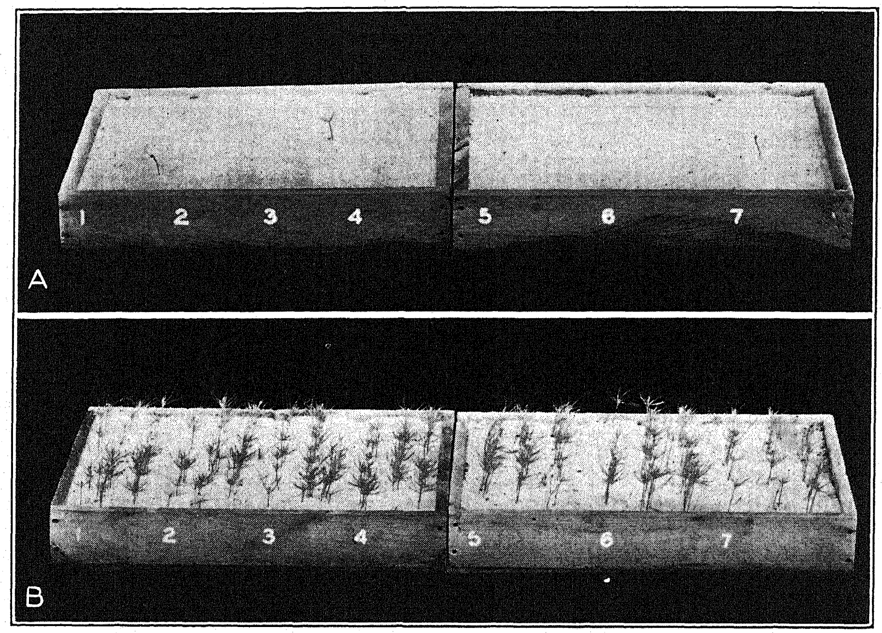

FIGURE 3. *Pinus taeda.* Seedling production in the greenhouse after storage for four years. Seeds mixed with varying amounts of CaO, and stored in refrigeration room. A. Planted directly in flats from storage flasks. (1) 100 grams seeds, and (2) 100 grams seeds +10 grams CaO, (3) 100 grams seeds+17 grams CaO, (4) 100 grams seeds+25 grams CaO, (5) 100 grams seeds +32 grams CaO, (6) 100 grams seeds +45 grams CaO, (7) 100 grams seeds+64 grams CaO. B. Planted in flat after one month in moist granulated peat moss at 5° C. Numbers as in A.

rable figures obtained after storage for five years were 4 and 50 per cent respectively. Seeds from which practically all the moisture was removed gave 7 per cent seedling production after two years' storage when planted immediately in greenhouse flats whereas a pre-treatment of seeds of the same lot for two months at 5° C. gave 39 per cent seedling production.

Corresponding figures obtained after five years of storage were 9 and 16 per cent (Table XII).

Unfortunately there was not a sufficient quantity of seeds stored sealed without CaO to continue tests after four years, but results up to that time indicated that these seeds were equal in vitality to the best of the dried ones and certainly better than those dried to excess.

Pinus taeda. In this form practically all of the moisture was removed without damage to the seed at least for two years of storage (Table XII).

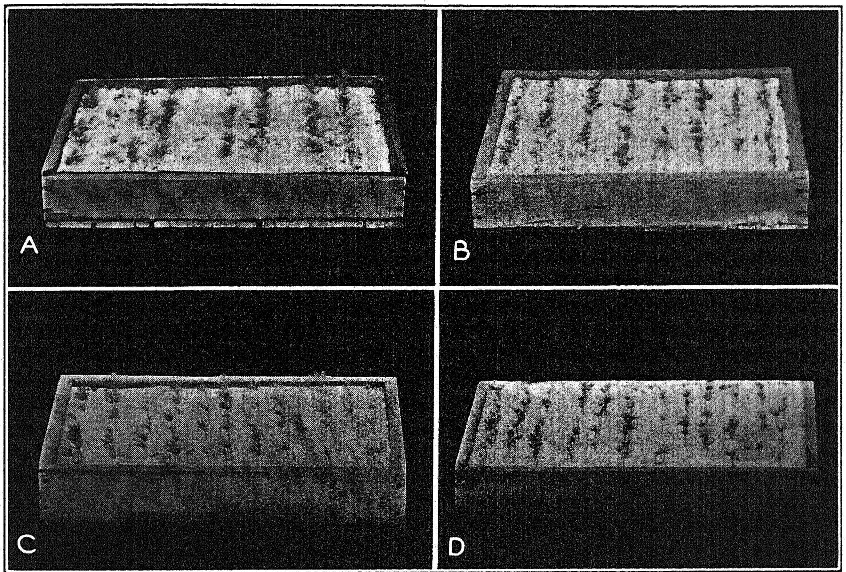

FIGURE 4. *Picea excelsa.* Seedling production in the greenhouse after storage. Seeds mixed with varying amounts of CaO and stored in refrigeration room. A and B stored one year. A. Planted directly in flat from storage flasks. B. Planted in flat after one month in moist granulated peat moss at 5° C. C and D stored four years. C as in A. D as in B.

Seeds from all storage conditions gave very satisfactory seedling production even after storage for five years, although there was some indication that excessive drying, i.e., mixing with twice the calculated amount of CaO to remove all moisture, is harmful after four years, and there is a further drop in seedling production from this storage condition after five years.

The effect of a low temperature pre-treatment on seedling production of this form is very marked in fresh as well as in stored seeds (usually difference of 50 to 60 per cent, Fig. 3).

Hence if one were testing the vitality of these seeds from a planting made directly in the greenhouse, he would conclude that the seeds were practically dead since they gave an average of about 3 per cent seedling

production, but a previous period at 5° C. results in a healthy stand of seedlings (50 to 70 per cent).

Pinus ponderosa. These seeds retained unimpaired germination capacity for four years under the conditions of this experiment (Table XII). In fact there seems to have been an increase in seedling production with storage. This "after-ripening" effect in storage has been noted by other workers. Unfortunately the quantity of stored seeds was exhausted after four years. In this form also can be seen the increase of the effectiveness of after-ripening with age.

Picea excelsa. Too much drying is harmful as seen by results of tests after four years. After five years seeds with no CaO were best while those with a small amount of CaO still retained their vitality very well. However, those with larger amounts of CaO had significantly decreased in germination power, especially in the ones dried most.

For stored as well as for fresh seeds of this form no pre-treatment is necessary for seedling production. In fact such pre-treatment tends to decrease rather than increase seedling stand.

Picea canadensis. Drying had no effect on keeping quality up to five years, at which time excellent seedling production was obtained from all conditions. Whereas low temperature pre-treatment did not seem effective for fresh seeds or for seeds after one year of storage, it became increasingly effective with storage and was most effective after five years when it increased germination from 21 to 88 per cent (dried seeds). The latter result was almost twice that obtained from fresh seeds.

SUMMARY

Experiments to determine the effects of sealing, temperature, vacuum, and desiccation on the keeping quality of seeds of some coniferous forms showed that sealed storage at low temperatures (5° C. or −5° to −15° C.) was effective for the maintenance of vitality. *Pinus taeda* seeds under sealed, low temperature storage retained their seedling-producing power fully for seven years, whereas in open room temperature storage, there was a decided decline in vitality after one year, and only a few seedlings were obtained thereafter. *P. caribaea*, *P. echinata*, and *P. resinosa* kept only slightly better in open storage at room temperature and exhibited the same beneficial effects of sealing at low temperatures. *Pinus palustris* lost vitality much more rapidly. Seeds from open storage at room temperature lost their germination power completely in one year. Even under sealed, low temperature storage there was a gradual decrease in seedling production to 50 per cent of the original after five years. *Pinus ponderosa*, *Picea excelsa*, and *Picea canadensis* stored only in refrigeration rooms with temperatures of −5° C. and −15° C. kept well for four to six years.

All seeds were thoroughly air-dried before the experiments were

started. Artificial desiccation whether moderate or excessive was found ineffective or harmful.

A vacuum proved favorable to retention of vitality when the seeds were stored at room temperature and both vacuum and low temperature storage overcame in part the injurious effects of drying.

Actual seedling production in greenhouse flats was taken as an index of vitality. Treatment of fresh or stored seeds of *Pinus taeda*, *P. caribaea*, and *P. echinata*, and to a less extent *P. ponderosa*, in moist granulated peat moss at 5° C. for one or two months prior to planting in the greenhouse resulted in increases in seedling production. Fresh seeds of *Pinus palustris* did not respond to such pre-treatment but as their vitality decreased, pre-treatment became necessary to give maximum seedling production. Seeds of *Pinus resinosa*, *Picea excelsa*, and *Picea canadensis* required no pre-treatment.

Results here reported indicate that sealed storage at a low temperature is effective for the maintenance of vitality of these coniferous seeds for considerable periods.

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GERMINATION OF DELPHINIUM SEEDS

Lela V. Barton

INTRODUCTION

Seeds of annual delphinium do not germinate if planted at high temperatures. Very poor germination is obtained if the temperature is above 20° C. This fact presents a problem to delphinium growers who wish to plant seeds in the tropics or in summer in the temperate zone.

According to the American Fruit Grower (Vol. 52, No. 2, p. 35, 1932) Borthwick found that lettuce seeds do not germinate at temperatures of approximately 30° C. and above. He was able to overcome this inhibiting effect of high temperatures by storing the seeds moist with good aeration at about 40° F. for from four to six days. This moist storage was accomplished by placing the seeds between folds of moist burlap and storing them on ice. The seeds were then dried at room temperature and planted.

The present tests were undertaken with a view to making possible seedling production of annual delphinium at high temperatures by pre-treatment at low temperatures. Tests were also made on seeds of the perennial form.

Seeds of 1931 crop were obtained from Vaughan Seed Company in the spring of 1932.

ANNUAL DELPHINIUM

TEST I

The first series was run so that the greenhouse plantings were made in August (temperature from 21° C. to 43° C. during the course of the tests). In this series lots of 100 seeds each were placed on moist filter paper in petri dishes for pre-treatment at 1°, 5°, or 10° C. or in a refrigerator (5° C. to 10° C.). Duplicate lqts of pre-treated seeds were removed from the filter paper and planted immediately in the greenhouse at intervals of from four days to four weeks. At the same time other petri dishes were transferred from the low temperatures to various higher temperatures in electrically controlled ovens. Both constant and daily alternating temperatures were used for germination.

The results are shown in Table I. It should be kept in mind that the figures represent *germination* (or the emergence of the hypocotyl) in the oven tests, while actual *seedling production* (or the appearance of the shoot above ground) is given in the case of the greenhouse plantings.

It is apparent that the higher temperatures (from 20° C. up) are decidedly unfavorable to the germination of annual seeds. Untreated seeds gave no germination at 25°, 30°, or 35° C. At 20° to 30° C. daily alternation or in the hot greenhouse small percentages (13 and 14 per cent) were obtained while the best germination (82 per cent) was obtained at 15° C.

TABLE I

EFFECT OF PRE-TREATMENT ON DELPHINIUM SEED AT LOW TEMPERATURES ON THEIR GERMINATION AT VARIOUS HIGH TEMPERATURES AND THEIR SEEDLING PRODUCTION IN THE GREENHOUSE

Germination environment	Pre-treatment	Percentage obtained after pre-treatment for									
		Annuals					Perennials				
		4 days	1 wk.	2 wks.	3 wks.	4 wks.	4 days	1 wk.	2 wks.	3 wks.	4 wks.
15° C.	1° C.	92	82	80	85	74	91	94	95	97	91
	5° C.	82	79	81	84	83	93	92	89	96	94
	10° C.	87	80	90**	—	—	95	89	98**	—	—
	Refrigerator	89	84	88	88	—	93	88	99	92	94
	Control	82					96				
20° C.	1° C.	68	55	47	66	60	98	93	95	93	91
	5° C.	69	75	60	67	74	94	89	94	97	91
	10° C.	76	74	81**	—	—	93	98	93**	—	—
	Refrigerator	74	73	80	72	—	95	91	92	92	92
	Control	62					95				
25° C.	1° C.	0	0	0	1	0	88	83	76	59	89
	5° C.	0	0	4	2	21	83	89	70	79	94
	10° C.	0	6	57**	—	—	72	85	96**	—	—
	Refrigerator	0	2	15	10	—	75	69	80	82	89
	Control	0					73				
30° C.	1° C.	0	0	0	2	3	55	58	56	34	9
	5° C.	0	0	0	0	5	58	72	70	60	54
	10° C.	1	2	41**	—	—	81	80	84**	—	—
	Refrigerator	0	0	14	34	—	73	60	60	38	35
	Control	0					66				
35° C.	1° C.	0	0	0	0	0	0	0	0	0	0
	5° C.	0	0	0	0	2	0	0	0	4	24
	10° C.	0	0	12**	—	—	0	0	37**	—	—
	Refrigerator	0	0	0	0	—	0	0	0	0	5
	Control	0					0				
20°–30° C.	1° C.	8	1	18	8	2	98	94	94	96	89
	5° C.	7	12	11	31	24	100	98	88	91	90
	10° C.	25	27	70**	—	—	96	95	95**	—	—
	Refrigerator	8	24	50	83	—	94	95	96	94	94
	Control	14					91				
Greenhouse*	1° C.	21	21	41	61	63	69	71	68	72	81
	5° C.	15	24	76	58	30	72	83	41	74	77
	10° C.	30	53	97**	—	—	83	69	27**	—	—
	Refrigerator	34	39	79	71	—	82	70	55	78	89
	Control	13					76				

* Greenhouse plantings made in August; temperature 21° C. to 43° C

** Split when transferred; some starting to germinate.

Effect of Pre-Treatment on Germination in Ovens

Germination at 25° C., 30° C., and 35° C. When seeds were pre-treated at various low temperatures the germination temperature was considerably extended (Table I). Germination was possible at 25° C. and 30° C.

and even at 35° C. when preceded by favorable treatment at low temperatures. One degree centigrade was ineffective. After four weeks at 5° C., 21 per cent germination was obtained at 25° C. There was germination at both 25° and 30° C. after two weeks in the refrigerator, and three weeks in the refrigerator made possible 34 per cent germination at 30° C. Pre-treatment for longer than three weeks in the refrigerator was not possible because the seeds began to germinate at the low temperature. This same condition was found in all of the low temperatures, the limit of time of pre-treatment being determined by the temperature. Germination was more delayed at 1° C. than at 5° C., 10° C., or in the refrigerator; hence longer pre-treatment was possible.

After pre-treatment for two weeks at 10° C., 57 per cent germination was obtained at 25° C., 41 per cent at 30° C., and 12 per cent at 35° C. These were the only seedlings obtained at 35° C. except 2 per cent after a pre-treatment of four weeks at 5° C.

In view of the results with untreated seeds at these high temperatures, results after pre-treatment were striking. The refrigerator and 10° C. were more effective than 1° C. or 5° C. in inducing germination at high temperatures.

Germination at 20° to 30° C. A daily alternating temperature of 20° to 30° C. was a good one for showing the effect of pre-treatment. This temperature was too high to permit much germination without pre-treatment, but at the same time it was low enough to permit good germination after proper pre-treatment. Twenty degrees centigrade to 30° C. also most nearly approximated the temperatures in the hot greenhouse. Again pre-treatment at 1° C. did not improve germination. A previous period at 5° C. for three or four weeks raised the germination from 14 per cent to 31 per cent and 24 per cent respectively. Treatment in the refrigerator for four days, and one, two, and three weeks gave a gradual increase in germination at 20° to 30° C. up to 83 per cent (8, 24, 50, and 83 per cent respectively). Good results were also obtained from pre-treatment at 10° C. where periods of four days, one week, and two weeks resulted in 25, 27, and 70 per cent germination at 20° to 30° C. Again the refrigerator and 10° C. were superior to 1° C. and 5° C. for pre-treatment.

Germination at 15° and 20° C. Good germination was obtained at constant temperatures of 15° and 20° C. without treatment (82 and 62 per cent). Germination at 15° C. was higher throughout the tests than at 20° C., but 20° C. proved fairly satisfactory. Since these temperatures were favorable for germination, pre-treatment at low temperatures had little or no effect.

Effect of Pre-Treatment on Seedling Production in Hot Greenhouse

This greenhouse (21° C. to 43° C. during the course of the test) gave practically the same seedling production from untreated seeds as was ob-

tained in the ovens at a daily alternating temperature of 20° to 30° C. Results from treated seeds were also practically the same in both of these conditions with the exception that 1° C. for four days to four weeks gave 21 per cent to 63 per cent seedling production in the greenhouse whereas the same treatment was ineffective for germination at 20° to 30° C. Five degrees centigrade for four days resulted in 15 per cent seedling production, while as a result of one and two weeks at this temperature 24 per cent and 76 per cent seedling production was obtained in the greenhouse. Periods of pre-treatment longer than two weeks decreased the yield. This same fact was noted for refrigerator and 10° C. treatments. Refrigerator and 10° C. produced the same general effect as 5° C. except that somewhat better seedling stands were obtained. The most effective pre-treatment was 10° C. for two weeks after which 97 per cent seedling production was obtained in the hot greenhouse. The pre-treatment period should be terminated before the seeds start to germinate since the transfer may injure the delicate young roots.

TEST 2

In the second series of tests a daily alternation of 20° to 30° C. alone was used for germination tests and a cool greenhouse (11° C. to 26° C.) was used for seedling production tests. Seeds for the oven tests were pre-treated as described for Test 1 with the addition of 15° C. as a pre-treatment temperature, but seeds for greenhouse tests were pre-treated on both moist filter paper and in soil in pots. The results are shown in Table II.

TABLE II

EFFECT OF PRE-TREATMENT OF DELPHINIUM SEED AT LOW TEMPERATURES ON THEIR GERMINATION AT 20° TO 30° C. AND THEIR SEEDLING PRODUCTION IN THE GREENHOUSE

Germination environment	Pre-treatment	Percentage obtained after pre-treatment for											
		Annuals						Perennials					
		Petri dishes			Pots			Petri dishes			Pots		
		1 wk.	2 wks.	3 wks.	1 wk.	2 wks.	3 wks.	1 wk.	2 wks.	3 wks.	1 wk.	2 wks.	3 wks.
20° to 30° C.	1° C.	8	3	1	—	—	—	92	95	83	—	—	—
	5° C.	21	40	27	—	—	—	92	95	93	—	—	—
	10° C.	19	54	—	—	—	—	99	96	—	—	—	—
	15° C.	70	—	—	—	—	—	—	—	—	—	—	—
	Refrigerator	18	24	—	—	—	—	94	95	—	—	—	—
	Control	17						93					
Greenhouse*	1° C.	61	55	49	54	37	43	77	71	82	80	78	82
	5° C.	68	61	70	46	40	35	83	87	70	58	86	80
	10° C.	68	63	—	36	42	32	86	84	—	67	87	68
	15° C.	38	—	—	37	28	53	—	—	—	57	88	61
	Refrigerator	64	53	—	52	51	44	86	88	—	39	86	73
	Control				70						84		

* Greenhouse plantings made in September and October; temperature 11° C. to 26° C.

Effect of Pre-Treatment on Germination at 20° to 30° C.

Results here were similar to those obtained in Test 1. It is of interest to note that pre-treatment at 15° C. for one week gave good results. This points to the possibility of eliminating the necessity of very low temperature chambers for pre-treatment.

Effect of Pre-Treatment on Seedling Production in Cool Greenhouse

It will be noted that untreated seeds gave 70 per cent seedling production in a cool greenhouse. No advantage was gained by pre-treatment. While slightly better crops were obtained if the seeds were treated on moist filter paper and then removed and planted in soil, direct planting in pots for the low temperature pre-treatment would probably prove a more practical method, when the seeds are to be germinated at high temperatures.

PERENNIAL DELPHINIUM

TEST 1

Although seeds of the perennial form were not nearly so sensitive to high temperatures as the annual seeds (Table I), the favorable effect of low temperature pre-treatment was evident when germination was at 35° C. No germination was obtained from untreated seeds at this temperature whereas pre-treatment of three and four weeks at 5° C. gave 4 per cent and 24 per cent; four weeks in the refrigerator gave 5 per cent; and two weeks at 10° C. gave 37 per cent. However, this is probably not significant practically since a constant temperature of 35° C. very rarely obtains. No advantage is gained by pre-treatment of perennial seeds to be planted in either a hot or a cool greenhouse (Tables I and II).

CONCLUSION

Seeds of the annual delphinium may be induced to germinate at high temperatures, which ordinarily are inhibitive, by pre-treatment for a short period in a moist condition at low temperatures. Since pre-treatment temperatures of 1° C. and 5° C. proved less effective than 10° C. or a refrigerator (5° to 10° C.) and since even 15° C. was found satisfactory, an ordinary cold storage room should suffice. The seeds may be pre-treated on a moist surface for one, two, or three weeks at temperatures of about 15° C., 10° C., or in a refrigerator, after which a good seedling stand can be obtained at constant temperatures up to 30° C. or in a greenhouse with a range in temperature of 21° C. to 43° C.

DORMANCY IN SEEDS OF BENZOIN AESTIVALE L.

ELTORA M. SCHROEDER

INTRODUCTION

Exposure to low temperatures for various lengths of time has been found to be effective in overcoming dormancy. Many rosaceous forms have responded to low temperature treatment (5, 6, 7, 8, 9). Barton (3) stated that pre-treatment for three months at 5° C. gave very good results for *Myrica carolinensis* Mill. Similar treatment was effective for seeds of coniferous trees (1, 2, 14).

Many seeds require a period of exposure to high temperature preceding low to overcome dormancy. *Tilia* (15) after-ripened with low temperature treatment. However, Barton (4) reported that a pre-treatment of either four months at 20° C. or 20 minutes' treatment of the extracted seeds with concentrated sulphuric acid was necessary to render the seed coat permeable before low temperature was effective. *Symphoricarpos racemosus* (11) and *Halesia carolina* (12) gave about the same response. Exposure to low temperature resulted in a good stand of seedlings in *Rhodotypos kerrioides* (10), but when these seeds were subjected to one month at 25° or 30° C. prior to low temperature treatment, they gave a much better response.

A progress report from the Michigan State College Experiment Station (13) stated that 20 per cent seedling production was obtained from fresh seeds of *Benzoin aestivale* L. planted in seed beds in the nursery in October. Greenhouse plantings made at the same time and two months later gave no results by July of the following year. These results indicate a dormancy of the seed.

The following experiments were conducted to ascertain the degree of dormancy in seeds of *Benzoin aestivale* L. and to determine how this dormancy may be overcome most effectively.

MATERIALS AND METHODS

The seeds were collected from the Boyce Thompson Arboretum, Yonkers, New York, September 27 and October 3, 1934. Each lot was cleaned the day after harvesting and spread to dry. The two sets of seeds were then combined and tests made from the resultant mixture.

All seeds put in the ovens for treatment were mixed with moist granulated peat moss. For general oven germination tests, lots of seeds were subjected to constant high temperatures in thermostatically controlled ovens and to daily alternating temperatures consisting of 16 hours at the lower temperature and 8 hours at the higher temperature. To find the proper temperature for pre-treatment, seeds were put in low temperature

ovens, sample plantings being made in the greenhouse after each month for six months. Similar lots were placed at 25° C. and transferred to low temperatures after various lengths of time. Sample plantings from this second lot were made every month after transfer to the low temperature until the seed supply was exhausted.

Plantings in flats consisting of duplicate lots of 200 seeds in each were made with the fresh seeds. Some of the flats were placed outside immediately while others were placed in the greenhouse. Greenhouse flats were removed to cold frames after one, three, and four months. For the outside plantings, frames covered with closely fitting sashes, referred to as board-covered frames, and frames in which the flats were covered with leaf mold under the board cover, referred to as mulched frames, were used. The mulched condition allowed a narrower range of temperatures than did the board-covered. It also had a higher average temperature as it was not as sensitive to the sudden temperature changes outside. Records of the mulched frame showed an average of from 5° to 8° C., whereas the temperature of the board-covered frame varied from −5° C. to 10° C., averaging about 0° to 2° C.

The soil used for all plantings consisted of a mixture of one-third sod soil, one-third peat, and one-third sand. The greenhouse temperature was 21° C. during the winter and spring, until the warm weather made it impossible to control the temperature.

This experiment was begun October 18, 1934, two to three weeks after collecting the seeds. Because of a shortage of seeds, the tests were made only when the seeds were fresh.

RESULTS

OVEN TESTS

No Pre-Treatment

The seeds in the general oven tests were left in the ovens until germination was complete. As will be seen from the data in Table I, the daily alternating temperature of 10° to 30° C. was optimum for germination (88 per cent) with 10° to 20° C. next (70 per cent). In spite of the fact that a high percentage was obtained, the germinations were scattered over a

TABLE I

PERCENTAGE GERMINATION OF BENZOIN AESTIVALE L. SEEDS IN THE OVENS; 100 SEEDS EACH LOT; DURATION OF EXPERIMENT, 6 MONTHS

Constant temperature				Daily alternation			
15° C.	20° C.	25° C.	30° C.	10° to 20° C.	10° to 30° C.	15° to 30° C.	20° to 30° C.
5	1	12	18	70	88	39	18

period of six months reaching the peak at about the beginning of the fifth month. Germination at the higher alternating temperatures and at the constant temperatures was poor. It is interesting to note that it was possible to provide an intermittent exposure which gave the seeds the low temperature treatment which they seem to require. This is in contrast to the continuous pre-treatment at low temperatures to be described below.

The coats were removed from the seeds completely or, in some cases, over the radicle only, in order to ascertain whether or not the coat was instrumental in preventing germination. However, these seeds showed no improvement over those with intact coats and this part of the experiment was discontinued.

Seedlings obtained from oven germinations of the intact and excised seeds were planted in the greenhouse. They appeared above the soil about a month later and were normal. The epicotyl is evidently not dormant.

Pre-Treatment

Low temperature. Lots of seeds were mixed with moist granulated peat moss and given low temperature treatment at 1°, 5°, and 10° C. Monthly samples were made in the greenhouse in order to determine the optimum pre-treatment period required for maximum seedling production.

Four or five months at 5° C. and four months at 10° C. (Table II) gave the best response. In those cases where 1° or 10° C. gave better results, the difference was not significant. There was a more prompt appearance of seedlings after treatment at 10° C. than at 5° C. However, the final percentages were about the same (Table II). Results from 5° C. were used in Figure 1 A which shows clearly the improved seedling production with increased time at low temperature.

TABLE II

EFFECT OF LOW TEMPERATURE PRE-TREATMENT ALONE ON SEEDLING PRODUCTION OF BENZOIN AESTIVALE L.; 100 SEEDS EACH SAMPLE

Temp. ° C.	Percentage seedling production after mos. of pre-treatment						
	0	1	2	3	4	5	6
1	—	5	19	35	28	51	17
5	—	12	33	66	78	81	—
10	—	4	21	52	81	48*	—
Control	1						

* 79 seeds used.

These seeds were evidently dormant, but this condition was overcome by exposure to constant low temperature (5° or 10° C.) in moist granulated peat moss for four or five months before planting. This produced a complete stand of seedlings within 32 days after planting as opposed to the

extended germination in the ovens when the low temperature was given intermittently over a period of six months (cf. germination at 10° to 30° C.). The seeds began to germinate in the ovens at 10° C. after four months and at 5° C. after six months. Germination did not occur at 1° C.

High temperature followed by low. Seeds mixed with moist granulated peat moss were subjected to a temperature of 25° C. for different lengths of time prior to low temperature treatment. Lots of these seeds were transferred to 5° C. after one-half, one, two, three, and four months, and to 10° C. after one and three months. Sample plantings in the greenhouse were made every month after removal to the low temperatures, except for the one-half month transfer. In this case the plantings were made at monthly intervals beginning one-half month after transfer to 5° C. (Table III).

TABLE III

EFFECT OF DIFFERENT PERIODS AT HIGH TEMPERATURE FOLLOWED BY DIFFERENT PERIODS AT LOW TEMPERATURE ON SEEDLING PRODUCTION OF BENZOIN AESTIVALE L.; 100 SEEDS EACH SAMPLE

Months at 25° C.	Low temp. ° C.	Percentage seedling production after mos. at low temp.											
		0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
0.5	5	1		2		39		89		85		70	
1	1		2		6		68		89		72		62
	5		5		22		90		87		88		—
	10		1		10		79		76		—		—
2	5		2		48		84		91		76		—
3	1		12		47		70		84		—		—
	5		6		48		86		91		—		—
	10		4		44		80		87		—		—
4	5		7		63		89		—		—		—

The percentage seedling production for the one-half and the one month transfers to 5° C. showed very little difference when the total pre-treatment period, including high and low temperature, was the same. For example, seeds treated for one-half month at 25° C. preceding three and one-half months at 5° C. gave the same results as those given one month at 25° C. prior to three months at 5° C. (Table III). The pre-treatment period in each case totaled four months. Therefore it will not be necessary to refer to the one-half month high temperature treatment in the discussion of the results. However, it was used in Figure 1 D to make the curve more complete.

As 5° C. was the most effective low temperature following a period at 25° C., data obtained from these tests were used in Figure 1 B, C, and D. Results of low temperature treatment without previous exposure to high

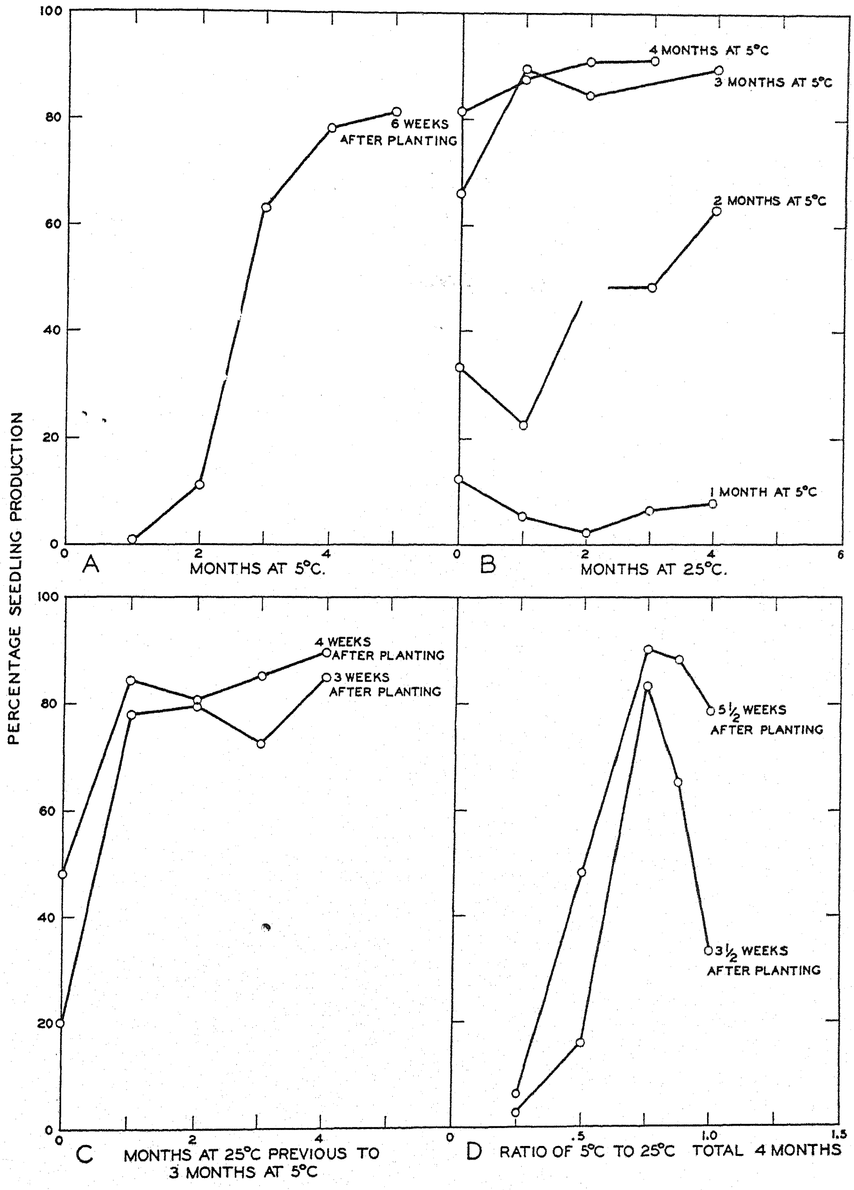

FIGURE 1. The effect of various lengths of pre-treatment on seedling production of *Benzoin aestivale* L. (A) shows that four months of pre-treatment at low temperature are required to obtain a high percentage of seedling production. (B) shows total seedling production using various combinations of periods at 25° and 5° C. Three months at 5° C. are necessary regardless of the period at high temperature. (B) and (C) show that this high temperature treatment need not exceed one month. (D) brings out the critical nature of the proportion of the four months allotted to low temperature.

were used as low temperature control, and greenhouse plantings of untreated seeds as general control (Table II).

Excellent seedling production resulted after three months at low temperature regardless of the length of exposure to 25° C. preceding it (Table III, Fig. 1 B). Three months at 5° C. without previous high temperature produced 15 per cent less seedlings (Table II). Figure 1 B also shows that further exposure to 5° C. after high temperature was unnecessary, but that one month less at 5° C. gave considerably inferior results. Dry seeds planted directly in the greenhouse produced one seedling. This was to be expected from the results obtained from the general oven tests described above, where those seeds subjected to high temperature alone gave poor results.

The total seedling production (Fig. 1 B) did not vary much with the different periods at 25° C. as long as three months of low temperature followed it. Since a three-month period is shown to be adequate, curves for seedling production after three and four weeks are given in Figure 1 C so that they may be compared with the total seedling production shown for this case in Figure 1 B. They show that the rate of seedling production is not affected by different periods at 25° C. as long as three months of low temperature follow. Thus from the point of time one month at 25° C. followed by three months at 5° C. was the optimum, a total pre-treatment of four months.

Next, it was necessary to find out whether or not the four months' pre-treatment was specific in its ratio of low to high temperature. Three months' exposure to 25° C. followed by one month at 5° C. was very poor. As the ratio of low temperature to high increased (Fig. 1 D), seedling production increased, reaching a critical point at 0.75 and 0.875 or three months at 5° C. preceded by one month at 25° C., and three and one-half months at 5° C. following one-half month at 25° C. respectively. However, the seedling production was considerably less when no high temperature was included in the pre-treatment period.

The greater effectiveness of the high-low temperature treatment over the constant low may have been due to an increased rate of water absorption resulting in a hastening of the changes which occur at low temperature.

Four months of exposure to high temperature (Table III) may shorten the period of low temperature required. Thus, two months of low temperature when preceded by four months of the high showed 63 per cent seedling production. This was an increase of 15 per cent over any other two-month low period preceded by a shorter period at high temperature, although it was not as good as constant treatment at low temperature for three months (Table II).

FLAT TESTS

One flat each was placed in the mulched and board-covered frames. At the same time, four flats were placed in the 21° C. greenhouse. One of these flats was left in the greenhouse throughout the entire experiment.

TABLE IV

RESULTS OF GREENHOUSE AND OUTSIDE PLANTINGS AFTER 9 MONTHS

Treatment		Percentage seedling production
Greenhouse (21° C.)		6
Cold frames	Mulched	79
	Board-covered	55
	Board-covered after 1 mo. in G.H. (21° C.)	69
	Board-covered after 3 mos. in G.H. (21° C.)	24
	Board-covered after 4 mos. in G.H. (21° C.)	69

The other flats were removed to the board-covered frame, one each after one, three, and four months and left there for the rest of the winter. The first seedlings of the outside plantings appeared about the middle of May. Final counts were made July 16, 1935, at the end of nine months.

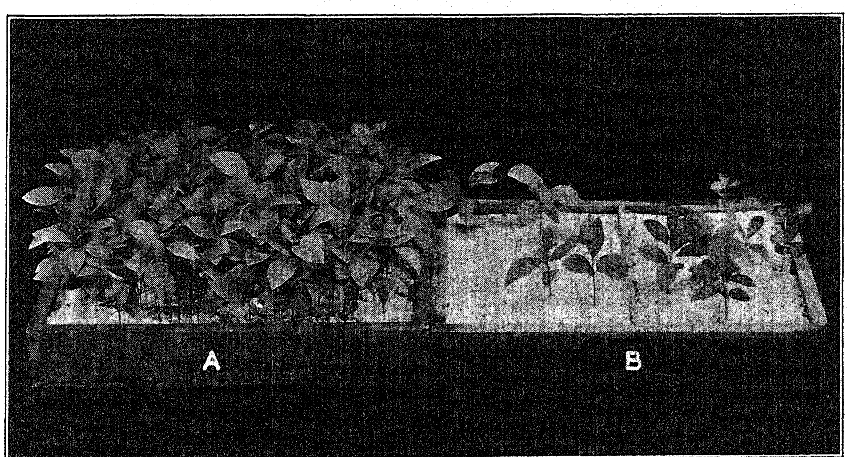

FIGURE 2. Seedling production of *Benzoin aestivale* L. planted October 18, 1934. (A) mulched throughout the winter; (B) in 21° C. greenhouse during entire experiment. Duplicate lots of 200 seeds each flat. Photographed July 3, 1935.

Seeds in the mulched flat (Table IV, Fig. 2 A) gave the best seedling production. The board-covered flat planting gave only 55 per cent, while seeds in those flats removed to the board-covered frame after one or four

months in the 21° C. greenhouse yielded 69 per cent. However, few seedlings were produced from those transferred to the board-covered frame after three months in the greenhouse. The average of all greenhouse controls for the nine-month period was 6 per cent, the individual plantings ranging from 1 to 12 per cent (Fig. 2 B).

These results confirmed fairly well those of the sample plantings. Constant low temperature produced a good stand of seedlings. The seeds in those flats placed directly in the mulched and board-covered frames may have had a short period of warm temperature before the winter set in since the plantings were made in October. The lot transferred in November showed an increase over that placed in the board-covered frame immediately. This difference was due to the effect of one month of high temperature in the greenhouse preceding the low temperature (between three and four months) of the board-covered frame. The February transfer was equally good. According to the results of sample plantings, when four months of high temperature treatment had been given, less low temperature was necessary. The low seedling production in the January transfer may have been due to the very low temperatures outside at the time of removal to the cold frame. As germination takes place only after four to six months in the low temperature ovens, there would be no danger of having the seedlings come through under the mulch.

SUMMARY

1. Optimum temperatures for germination of *Benzoin aestivale* L. in the ovens were daily alternations of 10° to 30° C. and 10° to 20° C. which gave 88 and 70 per cent respectively.

2. Four months in moist granulated peat at 5° or 10°C. before planting in the greenhouse resulted in a very good stand of seedlings.

3. The rate of seedling production and the total percentage were increased when the seeds were subjected to one month at 25° C. followed by three months at 1°, 5°, or 10° C. before planting. Two and three months at 25° C. were no more effective. If, however, the seeds were subjected to only two months at low temperature, at least four months at 25° C. preceding it were necessary.

4. The mulched frames gave better results than the board-covered frames. The effectiveness of the latter was greatly increased when preceded by one month in the 21° C. greenhouse.

5. Seedlings can be produced on a large scale by planting the fresh seeds outside in the fall and mulching them over the winter with no danger of losing seedlings under the mulch because of early germination. If fall plantings are not possible, seeds can be placed for four months in a refrigerated room (5° or 10° C.) in a moist medium before planting in the spring.

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HISTOPATHOLOGY OF NERVE LESIONS OF CICADA AFTER PARALYSIS BY THE KILLER-WASP

ALBERT HARTZELL

While the writer was engaged in the study of certain neuro-muscular poisons the effect of which may be detected in insects by the presence of lesions in the central nervous system (3, 7), an opportunity was afforded of obtaining specimens of adult cicadas paralyzed by the sting of the killer-wasp (*Sphecius speciosus* Dru.). The original material was received from Kentucky,[1] but later paralyzed specimens were obtained at Yonkers, New York, where the insect was abundant during August, 1933 and 1934. Its burrows were found in lawns and in between flagstones. The mounds of fresh earth in front of the burrow entrances at first suggested the work of field mice.

This large, formidable wasp preys upon the adult dog-day harvest fly (*Tibicen pruinosa* Say). It stings its victim and in the struggle that ensues both fall to the ground and the wasp seizing and straddling the cicada carries it back to the tree from which they fell to obtain a vantage point from which to fly in the direction of the burrow.

The burrows are usually made in sandy soil, especially in terraces along roadways. They slope gently for about six inches with side branches coming off at right angles at irregular intervals. The side branches continue for 6 or 8 inches farther and terminate in globular cells 1-1/2 inches in diameter. Each cell normally contains a cicada or occasionally two cicadas.

Riley (6) has given us a detailed description of the habits of this insect, but since the account was published many years ago and is not readily accessible a few details are repeated here.

According to the above author the egg of the wasp is deposited on the median femur of the cicada and in two or three days hatches into a whitish larva which completes its development in about a week. The larva possesses great extensile and retractile powers which enable it to penetrate and exhaust the body contents of its host. The larva hibernates in the cocoon made of earth held together by strands of silk and transforms to a pupa in the spring shortly before the appearance of the adult.

The sting of the wasp paralyzes the cicada rendering it into a comatose condition from which it is said never to recover. Physiological activity apparently goes on at a reduced rate. If the egg fails to hatch the paralyzed cicada is said to remain in a state of coma, in some instances for as long as a year. Material sent by mail from Owensboro, Kentucky, to Yonkers,

[1] The writer is indebted to Mr. Allan Reid of Owensboro, Kentucky, for specimens of paralyzed cicadas.

New York, in this study showed no evidence of decomposition that could be detected cytologically when compared with freshly-paralyzed specimens taken locally.

MATERIAL AND METHODS

The same technique was used in this work as has been previously described (3, 7). Particular attention was laid on the detection of lesions in the main parts of the central nervous system of the paralyzed cicadas.

In dissecting a longitudinal incision was made along the dorsal surface of the thorax and abdomen while the specimen was immersed in 95 per cent alcohol. The thoracic ganglia were removed with the aid of dental lances. The head was next dissected and the brain located by following the optic nerves to their source in the optic lobes. The location and removal of the suboesophageal ganglion is attended with difficulty although its position beneath the oesophagus aids in its location. The brain, suboesophageal ganglion, and thoracic ganglia were removed immediately after dissection and placed in separate vials containing 95 per cent alcohol.

The tissue was fixed in 95 per cent alcohol for 16 hours and stained for 5-1/2 hours with 0.1 per cent aqueous toluidine blue, following a technique used in medicine (4, v. 3, p. 651) for the detection of paralysis in humans. The tissue was washed in 95 per cent alcohol, dehydrated in absolute alcohol, and imbedded in paraffin after running through xylol. Gradual transitions were made by using combinations of xylol and alcohol and paraffin and xylol. Sections were of 5μ thickness.

The same technique was used to detect paralytic lesions in the tissue of the central nervous system of meal worms (*Tenebrio molitor* L.) which had been injected with formic and acetic acids.

EFFECT ON NERVE TISSUE

The central nervous system in the cicada is concentrated in the head and thorax to a greater extent than in more primitive insects. Because of this concentration the ventral abdominal ganglia are not very well developed. The nerve tissue from paralyzed cicadas showed areas that stained violet and other areas that were vacuolated with dark blue-stained margins. In the accompanying photomicrographs the violet-stained areas appear black. Lesions of this general description were found in the brain, suboesophageal ganglion, and the thoracic ganglia. The term tigrolysis is used here as in the medical literature to describe pathological degeneration of the Nissl granules.

Brain. Tigrolysis was especially well marked in the cortical region of paralyzed cicadas. Vacuolization of the tissue was noticeable also. The tissue of the check was not vacuolated and stained uniformly blue throughout. (Fig. 1, compare A and B.)

Thoracic ganglia. There was evidence of degeneration especially in the

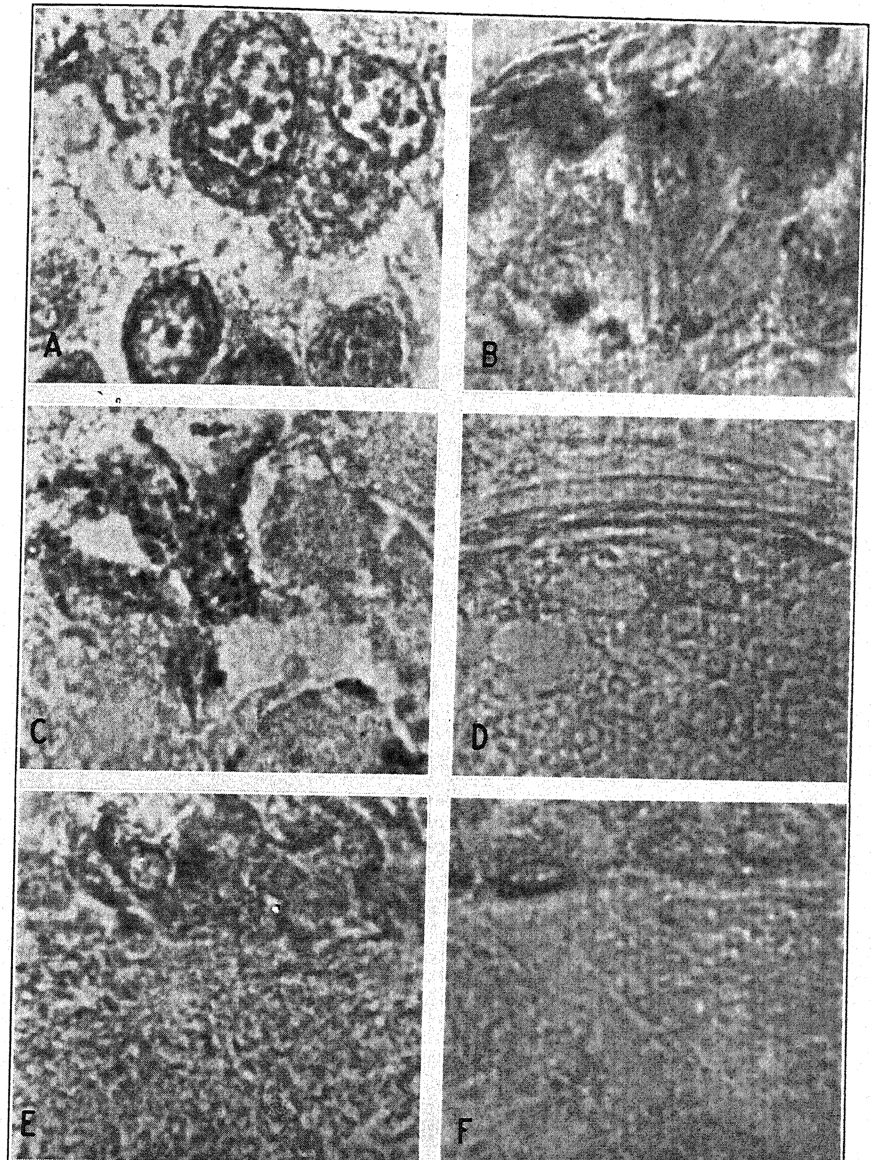

FIGURE 1. Magnification, 1600. Cross sections of nerve tissue of adult cicadas (*Tibicen pruinosa*) stained with toluidine blue. (A) Brain of paralyzed cicada. Note marked tigrolysis and vacuolization. (B) Brain of cicada killed by decapitation. (C) Thoracic ganglion of paralyzed cicada. Note disintegration and vacuoles. (D) Thoracic ganglion from individual killed by decapitation. Cross sections of ventral ganglia of meal worm (*Tenebrio molitor*). (E) Larva injected with formic acid. (F) Not injected. Neither tigrolysis nor vacuolization was produced by formic acid.

dorsal portion of the cortical region of paralyzed individuals. Tigrolysis and vacuolization of the tissue was less marked than in the brain. This contrasted with the uniform texture and blue-stained tissue of the check. (Fig. 1, compare C and D.)

Suboesophageal ganglion. In paralyzed specimens tigrolysis and vacuolization was much less marked than in the brain and thoracic ganglia.

A study of the abdominal ganglia and connectives was not made because of the difficulty of dissection due to the concentration of the nerve ganglia in the thorax. From the writer's experience in detecting nerve lesions caused by neuro-muscular poisons, such as triorthocresyl phosphate and the pyrethrins, paralyzed insects show lesions throughout the main parts of the central nervous system including the connectives (3).

ATTEMPT TO PRODUCE PARALYTIC LESIONS BY INJECTION WITH ORGANIC ACIDS

It was formerly surmised that formic acid was the chief constituent of the venom of the honeybee (*Apis mellifica* L.) and other stinging Hymenoptera. Merl (5), in 1921, by carefully conducted experiments proved that bee venom does not contain even a trace of formic acid although its exact chemical composition still remains unknown. On the other hand it is definitely known that the venom of ants contains formic acid, and produces an effect similar to that of bee venom, but not nearly so marked. The constituents in honeybee venom consist of one with an acid reaction and another with an alkaline reaction. It is claimed that both are necessary for their deadly effect and that in insects which simply paralyze their prey, as among the solitary wasps or Eumenidae, the alkaline glands are functionless (2, p. 64). All that is definitely known regarding bee venom is that it contains a toxic protein-like substance. The active principle is believed to be in combination with lecithin, and related to certain constituents of snake venom and cantharidin (1, p. 37–43). The amount of venom injected by an insect at one time is evidently quite variable, and always in small doses, a mere droplet. In the case of the honeybee it is generally believed, but on limited direct evidence, that the amount of poison is greatly increased at times, the period of the buckwheat honeyflow being one of the worst for severe stings. The sting of the killer-wasp is known to be of unusual severity to humans.

In the present investigation an attempt was made to induce paralysis by injections of formic acid and acetic acid. Five meal worms (*Tenebrio molitor*) injected with 0.06 cc. of 5 per cent formic acid and another group injected with a similar amount of 10 per cent acetic acid showed symptoms of local paralysis, but the central nervous system of such larvae when dissected and stained with toluidine blue did not exhibit nerve lesions that in any way resembled those found in the tissue of the central nervous

system of paralyzed cicadas that were stung by the killer-wasp (Fig. 1, E and F). Injection of larvae with higher concentrations of these acids produced results comparable to those obtained with lower concentrations. This lends cytological support to Merl's contention that the venom of stinging Hymenoptera does not contain formic acid.

SUMMARY

Adult cicadas (*Tibicen pruinosa*) paralyzed by the sting of the killer-wasp (*Sphecius speciosus*) showed nerve lesions in the main parts of the central nervous system when the tissue was stained with 0.1 per cent aqueous toluidine blue, following a technique used in medicine for the detection of paralysis in humans.

The lesions exhibited a general similarity to those produced in the nerves of insects killed with triorthocresyl phosphate and with the pyrethrins.

Attempts to produce similar paralytic lesions in meal worms (*Tenebrio molitor*) by injections with formic and acetic acids resulted negatively.

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EFFECT OF LIGHT INTENSITY ON THE MANGANESE CONTENT OF PLANTS

M. M. McCool

It was reported (3) that steaming under pressure caused six soil types to be less productive for several kinds of plants. The retardation of growth was least under conditions of low light intensity and the harmful effects were nullified by the addition of fertilizer. The water soluble manganese content of the soils was markedly augmented by steaming and large amounts of this element were present in leaves of plants grown in the soils so treated.

Additional studies of soil, manganese, and plant relationships have been conducted. In this report, however, are given only the results obtained from investigations concerned with the effect of light intensity on the deleterious action of manganese and on the amount of this element taken up by leaves of soybean (*Glycine max* Merr.) and buckwheat (*Fagopyrum esculentum* Moench.), which were grown outside the greenhouse. More elaborate experiments were conducted in a greenhouse with soybean, snap bean (*Phaseolus vulgaris* L., var. Tendergreen), and Turkish tobacco (*Nicotiana tabacum* L.).

MATERIALS AND METHODS

Plants in the outside experiments were grown in glazed jars of two-gallon capacity, filled with Podunk silt loam. Manganese sulphate ($MnSO_4 4H_2O$) was employed as the carrier of manganese. The salt was thoroughly mixed with the soil before seeding. The containers, with certain exceptions, were partially buried so that their surfaces were on a level with that of the surrounding soil. The soil in the jars was watered after suitable intervals of time with distilled water. The seeds were planted in a row and boards were placed on the jars so that an opening about one-fourth of an inch wide remained. These arrangements reduced the amount of distilled water required and temperature variations of the cultures.

The soil cultures were placed in the open and in the large cloth-covered enclosures described by Arthur and Stewart (1). One giving low light intensity was covered with unbleached muslin which transmitted 35 per cent of the total solar radiation; the other gave medium light intensity through cheesecloth which transmitted 58 per cent of the total solar radiation. Rain was excluded from the cultures by placing glass frames over them as needed.

Glazed jars of two-gallon capacity filled with Gloucester loam were utilized in the greenhouse tests. One set of cultures was untreated and the other received manganese sulphate. The containers were covered as were

those in the experiments described above and in addition boards were so arranged that the rays of the sun did not strike the jars. The cultures were placed in the greenhouse and in three enclosures or cages each of the dimensions of which was three feet. Two of them were covered as were those in the open, and the additional enclosure was covered with cheesecloth which transmitted 78 per cent of the solar radiation. The conditions under which the plants were grown are spoken of in the text as greenhouse or unshaded, lightest shade, medium shade, and heavy shade. Each cultural treatment was replicated four times.

The manganese content of the leaves, and in some instances that of known leaf areas, was ascertained. Thirty or more leaves of soybean plants were collected and their total area determined by means of blue prints and a planimeter. A cork borer was used in sampling the bean and tobacco leaves. All were finely ground before samples were taken for analysis.

RESULTS

OUTSIDE EXPERIMENTS

The outside experiments were conducted during the summers of 1933 and 1934. The containers were not sunken in the ground in 1933 and consequently there were wide differences in the temperature of the soil under the different growth conditions as the results in Table I show. The tem-

TABLE I

TEMPERATURE IN ° F. OF AIR AND SOIL CULTURES UNDER DIFFERENT INTENSITIES OF LIGHT

Growth condition	Air	Soil in jars three inches from surface					
		Adjacent to rim		One inch from rim		Center of jar	
		*	**	*	**	*	**
Outside	91.0	104.0	85.0	102.0	81.8	97.0	79.2
Greenhouse+medium shade	91.6	97.0	80.0	96.0	78.5	91.5	79.0
Greenhouse+heavy shade	91.5	92.0	81.5	91.4	79.0	91.4	77.8

* Containers placed on the surface of the ground.
** Containers partially buried.

peratures of the air and of the soil in the jars placed under different light intensities were determined at 1:30 p.m., July 3, 1934. It should be noted that the temperature of the soil in the containers which were placed on the surface of the ground varied widely under the different conditions of the experiment. Differences in the temperatures of the soil in the partially buried and covered jars were strikingly less.

Experiments with soybean and buckwheat. Soybean and buckwheat seed were planted in soil cultures June 26, 1934, and were harvested 25 days later. About six days after the soybean plants appeared the leaves gave

evidence of manganese injury. They were smaller in size and lighter green in color than were those grown in the untreated soil and in addition brown spots were present on them. At the time of harvest the injury to the plants in the cultures which had received manganese sulphate at the rate of 500 parts per million parts of soil varied somewhat with the light intensity under which they were grown. The growth of the plants which had been outside the cages was retarded greatly, the leaves were small, shriveled, somewhat chlorotic, and many brown spots were present on them. The leaves of those produced under medium light intensity were slightly larger, the young leaves yellowish-brown in color, and numerous brown spots were present. The leaves of those produced under the lowest light intensity were visibly less injured than were those just discussed.

TABLE II

Effect of Light Intensity on the Yield and Content of Manganese in Soybean. Duration of Growth Period 25 Days

Treatment	Degree of shading					
	None		Medium		Heavy	
	Dry wt. in grams, 20 plants	Per cent manganese	Dry wt. in grams, 20 plants	Per cent manganese	Dry wt. in grams, 20 plants	Per cent manganese
No treatment*	9.3	0.038	10.0	0.034	7.5	0.039
No treatment**	9.6	0.049	10.7	0.041	7.1	0.030
500 parts of $MnSO_4 4H_2O$ in one million parts of soil*	5.6	0.511	6.0	0.391	5.4	0.410
500 parts of $MnSO_4 4H_2O$ in one million parts of soil**	6.3	0.538	6.1	0.444	5.8	0.455
250 parts of $MnSO_4 4H_2O$ in one million parts of soil*	7.3	0.257	7.3	0.234	6.0	0.230

* Containers placed on the surface of the ground.
** Containers partially buried.

The plants taken from the cultures to which 250 parts of manganese sulphate were added to one million parts of soil, were injured, the harmful effects being most obvious in the case of those grown in the open, followed in turn by those produced in the medium and low light intensities.

According to the yields of dry plant material, as given in Table II, the cultures which received 500 and 250 parts of manganese sulphate per million parts of soil respectively, produced in the open 54.6 and 78.2 per cent, in medium shade 60.0 and 72.8 per cent, and in heavy shade 72.0

and 80.0 per cent as much as the untreated ones under corresponding conditions. The yields derived from the control cultures under medium shade were slightly greater and much less under the heavy shade than were those from the unshaded cultures. The plant material produced in the partially buried cultures under medium shade to which 500 parts per million of manganese sulphate were added was 15 per cent greater than that obtained from the corresponding ones in the open, whereas the yield derived from those under the heavy shade was 5.6 per cent greater.

The results obtained from the buckwheat series of cultures are likewise of interest. The leaves of the plants grown in the soil which received the heaviest application of manganese and which were unshaded, were small, light in color, and curled at the edges. The effects on those grown in medium shade were less striking, and the visible injury to the leaves of the plants grown in the soil treated with the same amount of manganese sulphate and under heavy shade was slight.

TABLE III

EFFECT OF LIGHT INTENSITY ON THE YIELD AND MANGANESE CONTENT OF BUCKWHEAT. DURATION OF GROWTH PERIOD 25 DAYS

Treatment of Podunk soil	Degree of shading					
	None		Medium		Heavy	
	Dry wt. in grams, 20 plants	Per cent manganese	Dry wt. in grams, 20 plants	Per cent manganese	Dry wt. in grams, 20 plants	Per cent manganese
No treatment	9.4	0.078	8.0	0.050	5.0	0.046
500 parts of $MnSO_4 4H_2O$ in one million parts of soil	5.6	1.212	5.2	1.020	3.5	0.926
250 parts of $MnSO_4 4H_2O$ in one million parts of soil	7.3	0.603	6.4	0.562	4.4	0.500

The dry weights of the above-ground portions of the buckwheat plants taken from the different cultures are summarized in Table III. According to these data, the yields of dry matter obtained from the cultures, which received manganese sulphate in the ratio of 500 parts per million of soil, in the open, under medium, and low light intensities, were 60.1, 65.0, and 69.6 per cent of the corresponding controls and those harvested from the soil which received manganese sulphate in the ratio of 250 to one million were 77.7, 80.5, and 88.0 per cent of their controls. The yields derived from the various cultures decreased as the light intensity decreased but those produced in the manganese-treated soil declined slightly less than they did in the untreated soil.

Manganese content of leaves of plants. The manganese content of the

leaves of the plants taken from the various cultures was determined. According to the data in Table II the differences in the amount of this element in the leaves of the control soybean plants were negligible. The leaves of unshaded soybean plants grown in the manganese-treated soil, without exception, contained more manganese, dry weight basis, than did those of the other plants. The manganese content of the leaves of the plants grown in the containers which were on the surface of the ground was greater than it was in those which were collected from the partially buried containers. It appears, therefore, that the temperature of the soil affected somewhat the percentage of this element in the leaves of soybean.

According to the results presented in Table III, the manganese content of the leaves of buckwheat plants decreased as the light intensity under which they were grown became less. It should be noted also that the manganese content of the leaves of buckwheat plants was much higher throughout than was that of the leaves of the soybean plants.

GREENHOUSE EXPERIMENTS

Experiments with soybean. Soybean seeds were planted in cultures September 21, 1934, and harvested October 21, 1934, a season of the year of relatively low light intensity. The cultures were placed in the greenhouse and in cages providing medium and heavy shade. The plants grown in the soil cultures, to which were added 400 parts of manganese sulphate per million and which remained uncovered in the greenhouse, were severely injured, as evidenced by the yellowish-green color of the leaves and numerous brown spots on them. The plants in the manganese-treated cultures which were placed in the cage of medium shade were less severely injured and those in the heavily shaded cage showed very slight injury. Shading did not affect the untreated soil cultures. The yields derived from the manganese-treated and unshaded soil cultures were 11.2 per cent less than were those produced in the cages, as shown in Table IV.

TABLE IV

Effect of Manganese on the Growth of Soybean.
Duration of Growth Period 28 Days

Growth condition	Dry weight in grams, 20 plants	
	Control	Soil+400 p.p.m. manganese sulphate
Greenhouse	4.8	3.2
Greenhouse+medium shade	4.8	3.6
Greenhouse+heavy shade	4.6	3.6

The data involving the manganese contents of samples of the leaves and stems are given in Table V. The percentage of manganese in the first leaves to form diminished as the degree of shading under which the plants

TABLE V

MANGANESE CONTENT OF SOYBEAN LEAVES AND STEMS

Growth condition	1st leaves		2nd leaves		3rd leaves		Composite sample of leaves	Stems
	Per cent manganese	Mg. manganese in 100 sq. cm.	Per cent manganese	Mg. manganese in 100 sq. cm.	Per cent manganese	Mg. manganese in 100 sq. cm.	Per cent manganese	Per cent manganese
Greenhouse	0.350	6.15	0.230	3.13	0.127	0.16	0.315	0.112
Greenhouse+medium shade	0.320	3.89	0.251	3.19	0.126	0.20	0.281	0.119
Greenhouse+heavy shade	0.121	1.65	0.191	2.16	0.130	0.14	0.255	0.098

were grown decreased. The effect of light intensity, however, was more striking when the manganese content of equal areas of the leaves were considered.

There was less manganese in the second set of leaves to form under heavy shade than there was in those produced in the greenhouse and in the container of medium light intensity, whether the results are expressed on the basis of dry matter or equal areas of tissue. The third or youngest sets of leaves varied but slightly with respect to their manganese contents. The percentage of manganese in composite samples of the three sets of leaves became less as the light intensity under which the plants grew was decreased. The manganese content of the stems of the plants grown under heavy shade was slightly less than it was in the stems of the plants taken from the greenhouse and the enclosure of medium shade.

TABLE VI

Effect of Light Intensity on the Yield of Snap Bean. Duration of Growth Period 31 Days

Growth condition	Dry weight in grams, leaves and stems (15 plants)	
	Control	Soil+400 p.p.m. manganese sulphate
Greenhouse	12.2	8.8
Greenhouse+lightest shade	12.6	11.2
Greenhouse+medium shade	11.8	10.4
Greenhouse+heavy shade	9.6	8.8

Experiments with snap bean. Snap beans were grown in the greenhouse and in cages which provided light, medium, and heavy shade. The manganese sulphate was added at the rate of 400 parts per million of soil. The seeds were planted May 24 and the plants were harvested June 25, 1935. Unlike soybean there was no apparent injury to the first leaves by manganese. The second and third sets of leaves of the plants in the greenhouse and in the cage of the lightest shade were yellowish-green in color with brown areas on them. The effects on those in the cage which provided medium shade were not so outstanding, and there was no visible injury to those in the heaviest-shaded container.

As the data in Table VI bring out, the yields of dry material in the control cultures were somewhat less in the cages providing medium and heavy shade than they were in the greenhouse. The amounts of plant material derived from the manganese-treated soil cultures were greater in the cages of lightest and medium shade than were those from the greenhouse and heavily shaded cage.

According to the results given in Table VII the percentage of manganese in the leaves of the plants grown in the greenhouse was somewhat

higher than it was in those taken from the plants in the cages. It is to be observed, however, that the manganese content of equal areas of the leaves from the plants in the manganese-treated cultures decreased consistently and strikingly as the light intensity became less. The percentage of this element in the stems which were produced under the different growth conditions did not vary.

TABLE VII

EFFECT OF LIGHT INTENSITY ON THE MANGANESE CONTENT OF LEAVES AND STEMS OF SNAP BEAN

Cultural condition	Per cent manganese in leaves	Per cent manganese in leaf discs	Dry wt. in grams of 100 sq. cm. of leaves	Mg. manganese in 100 sq. cm. of leaves	Per cent manganese in stems
Greenhouse	0.227	0.215	0.342	0.735	0.037
Greenhouse+lightest shade	0.160	0.152	0.270	0.410	0.041
Greenhouse+medium shade	0.172	0.150	0.247	0.370	0.034
Greenhouse+heavy shade	0.133	0.143	0.144	0.205	0.031

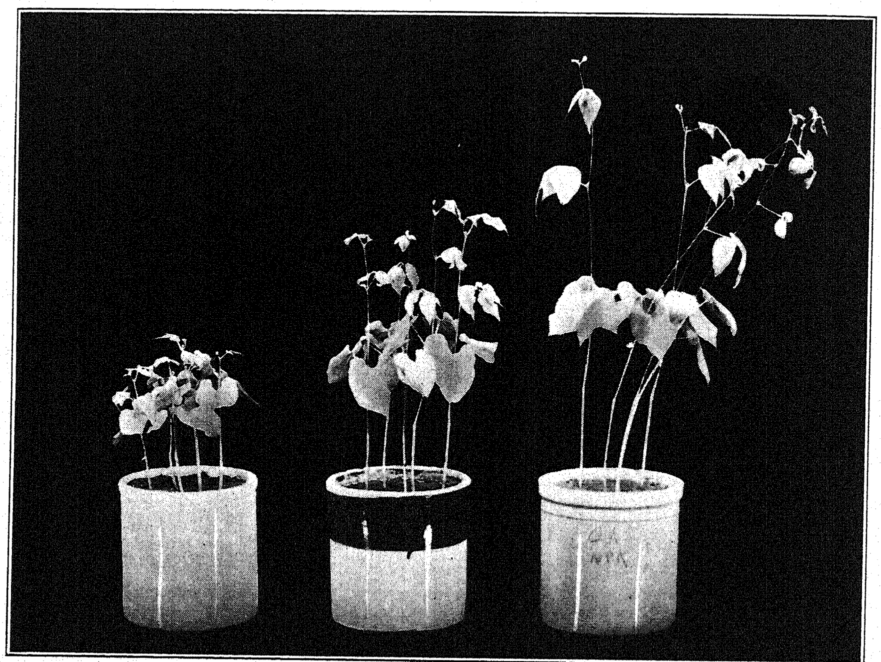

FIGURE 1. Snap beans in soil treated with manganese sulphate at the rate of 600 parts per million. Left to right: cultures from greenhouse, greenhouse+medium shade, and greenhouse+heavy shade.

In another set of soil cultures to which 600 parts per million of manganese sulphate were added the injury to the second and third leaves was discernible under each of the growth conditions, although it was less

TABLE VIII

Effect of Light Intensity on the Yield and Content of Manganese in Snap Bean. Duration of Growth Period 32 Days

Growth condition	Dry weight in grams, 15 plants				Per cent manganese		
	Leaves	Stems	Roots	Total	Leaves	Stems	Roots
Greenhouse	2.4	4.6	2.9	9.9	0.995	0.219	0.689
Greenhouse+medium shade	2.1	3.8	2.0	7.9	0.833	0.245	0.740
Greenhouse+heavy shade	1.8	2.8	1.4	6.0	0.813	0.297	0.735

striking in case of those grown in the heavily shaded cage as illustrated by Figures 1 and 2. The yields obtained and the percentage of manganese in the leaves, stems, and roots are given in Table VIII. The dry

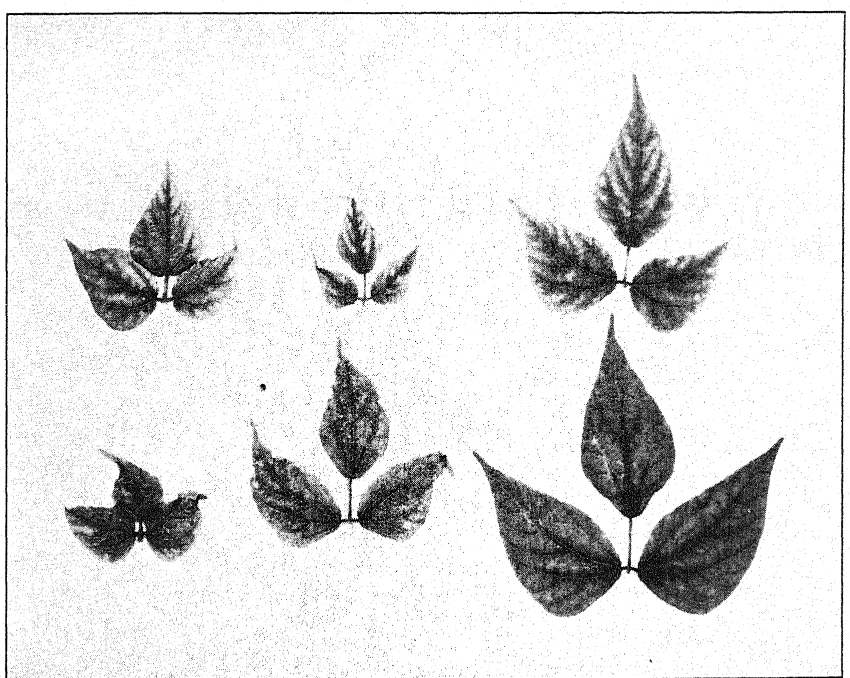

Figure 2. Second and third leaves of snap beans from soil cultures treated with 600 parts per million of manganese sulphate. Top: third leaves. Bottom: second leaves. Left to right: leaves taken from plants grown in the greenhouse, greenhouse+medium shade, and greenhouse+heavy shade.

weights of the plant materials harvested decreased as the degree of shading increased. It is to be noted also that the percentage of manganese in the leaves decreased as the light intensity under which the plants were grown became less, whereas the reverse was true with respect to the stems. There was slightly less of this element in the roots taken from the plants grown in the greenhouse than there was in those from the shaded containers.

Experiments with tobacco. Tobacco plants were grown from June 26 until July 31, 1935 in the greenhouse and in the cages of light, medium, and heavy shade. The yields derived from the control and manganese-treated cultures are given in Table IX. There was no evidence of manganese injury to the plants in the heavily shaded cage. It was severe with respect to those in the greenhouse and in the cage providing lightest shade, as evidenced by the low yields and chlorosis of the leaves; but the injury to those in the cage of medium shade was less apparent. These results are in accord with those obtained by the writer with Canadian field pea, in solution cultures, grown in a greenhouse and in a dark chamber (2).

TABLE IX

EFFECT OF LIGHT INTENSITY ON THE YIELD OF TOBACCO PLANTS. DURATION OF GROWTH PERIOD 35 DAYS

Growth condition	Dry weight in grams, leaves and stems (6 plants)	
	Control	Soil+400 p.p.m. manganese sulphate
Greenhouse	20.0	14.6
Greenhouse+lightest shade	18.5	12.0
Greenhouse+medium shade	21.7	18.2
Greenhouse+heavy shade	17.8	18.3

The manganese content (on the basis of dry matter) of leaves, stems, and roots and also of equal areas of leaves is given in Table X. The high manganese content of the leaves and roots of the plants produced in the greenhouse should be noted. Furthermore, marked decreases in the

TABLE X

EFFECT OF LIGHT INTENSITY ON THE MANGANESE CONTENT OF TOBACCO

Cultural condition	Leaves		Stems	Roots
	Per cent manganese	Mg. manganese per 100 sq. cm.	Per cent manganese	Per cent manganese
Greenhouse	0.993	2.990	0.178	1.111
Greenhouse+lightest shade	0.919	2.660	0.256	1.042
Greenhouse+medium shade	0.917	2.930	0.172	0.645
Greenhouse+heavy shade	0.652	1.270	0.164	

quantity of this element present in equal areas of leaves and in the roots resulted from medium and heavy shading of the plants.

SUMMARY

The effect of light intensity on the injurious action of manganese on soybean and buckwheat was studied outside and on soybean, snap bean, and tobacco in a greenhouse. The cultures employed in the former were placed in the open and in large enclosures which transmitted 35 and 58 per cent of the total solar radiation, and those which comprised the greenhouse experiments were placed in the greenhouse and in inclosures which transmitted 35, 58, and 78 per cent of it.

The visible injury (indicated by brown spots on and chlorosis of the leaves) to the plants grown in the manganese-treated soil decreased as the light intensity became less. With the exception of tobacco plants, the decrease in yields resulting from the addition of manganese sulphate to the soil was not prevented by shading the plants.

The percentage of manganese in the leaves of the soybean and buckwheat plants from the outside experiment decreased consistently as the light intensity decreased.

The manganese content of the leaves of soybean plants grown in the greenhouse decreased as the degree of shading increased as did that of equal areas of the first leaves to form. The effect of shading on the amount of this element in equal areas of the second and third leaves was less marked. The manganese content of equal areas of snap bean and tobacco leaves decreased as the intensity of the light under which they were grown became less. The percentage of manganese in the stems of the plants grown in soil treated with 400 parts per million of manganese sulphate varied but slightly, but it increased as the degree of shading increased in those grown in the soil which received 600 parts per million. The manganese content of the roots of the snap bean and tobacco plants was remarkably high, although shading decreased it in the tobacco plants.

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THE RESPONSE OF ROOTS TO "ROOT-FORMING" SUBSTANCES

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Several reports from this laboratory have concerned the responses of stems and leaves of plants to the so-called "hormones" or "growth substances." For example, synthetic chemical compounds were shown to induce epinasty of leaves, proliferations, swelling, bending and retardation in elongation of stems, and initiation of roots on stems and leaves (1, 5). Roots were not tested because they were more or less inaccessible for this type of experimental work. Our needs have recently been supplied by a grape vine which produces aerial roots when grown in the greenhouse. This report therefore concerns the response of roots to growth substances.

The following synthetic compounds were used in the tests: α-naphthaleneacetic acid, indolebutyric acid, indoleacetic acid, indolepropionic acid, Δ-(3-indolyl)-valeric acid,[1] and phenylacetic acid. The concentration range when dissolved in water was 0.00007 per cent to 0.0006 per cent; as lanolin preparations the concentration range was 0.01 per cent to 1.0 per cent. The roots were immersed in the water solutions, but the lanolin preparations were applied with a glass rod to the tips or along the side of the roots.

There is an unnamed species[2] of tropical grape (*Vitis* sp.) (3) which, when grown in a greenhouse, climbs to the roof and then spreads out, clinging to girders and other objects which the tendrils can grasp. Aerial roots grow from the nodes of the vines at frequent intervals and extend great distances without branching until they come in contact with soil or other moist material. In the Missouri Botanical Garden Conservatory, the roots grow for distances of 30 to 40 feet without branching (Fig. 1). According to Moore (3), who supplied cuttings for this laboratory, "the internal structure of the small roots is just like any other small root."

At the Boyce Thompson Institute a plant set in two cubic feet of soil grew vigorously, producing vines more than 20 feet in length. As these clung to the roof they produced aerial roots which soon touched the greenhouse beds ten feet below. No branching of the roots occurred until the tips came in contact with moist material, such as soil or water. Roots which were immersed in water produced several branches which in turn also branched if left for several days in water. When roots were cut off, one or two branches arose from the remaining stumps. Frequently more than one started but only one continued.

[1] All the indole compounds used in these experiments were synthesized and furnished by Dr. R. H. Manske of the National Research Council, Ottawa, Canada.

[2] This plant is now known to be *Cissus sicyoides* L. var. *Jacquini* Planchon.

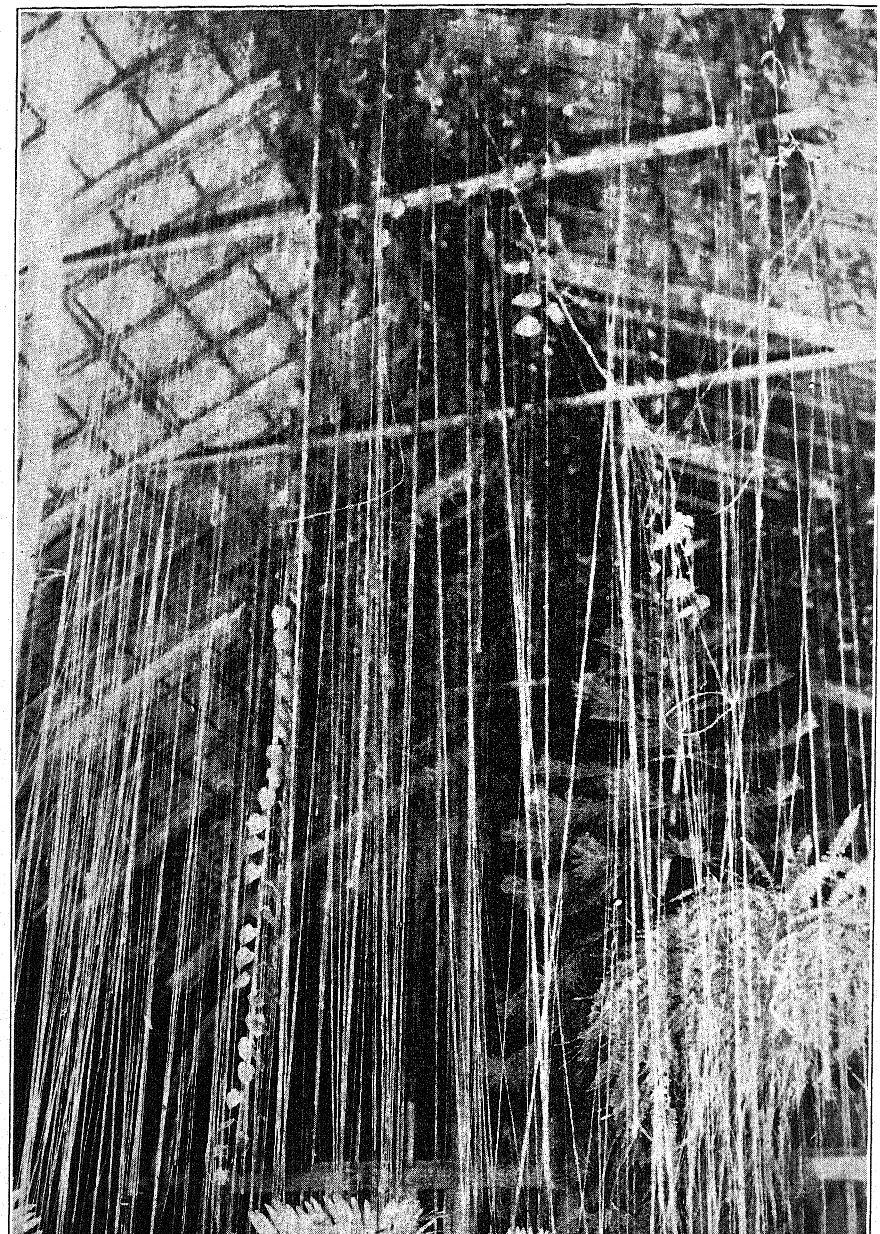

FIGURE 1. Tropical grape vine in the Cycad house at Missouri Botanical Garden showing aerial roots 40 feet long without branching until they come in contact with the ground. (Courtesy Missouri Botanical Garden.)

The average elongation per day of ten measured roots was four inches. The greatest elongation for any one root was six inches. The region of elongation as shown by increase in distance between millimeter marks stamped along the side of the root extended 10 or more inches back of the tip. The region of greatest elongation extended from the tip back two to three inches. For severed roots the elongation decreased as the distance back of the tip, where the cut was made, increased. Roots with only one-quarter inch of the tip removed made practically as much elongation during the first 24 hours as intact roots, while roots with six inches removed showed very little elongation. The young part of the root was very flexible while the older part was easily broken. The diameter which varied around one-sixteenth of an inch was nearly uniform throughout the entire length of a root.

The first noticeable responses of roots treated with lanolin preparations of growth substances over the region of elongation were bending, retardation in extension, and increase in diameter. These are similar to the response of etiolated stems of sweet pea and bean seedlings previously reported (5). Within three days after treatment newly initiated branch roots could be seen emerging through the epidermis. These continued to elongate until they were approximately one-eighth inch in length, at which time the tip of the main root appeared to recover from the treatment and renewed its growth. As soon as the rate of growth at the tip approached normal the new side roots were completely inhibited. They were, however, released again if the tip of the main root was re-treated with growth substance. After they reached one-half inch or more in length they appeared to be freed from further influence of the main root tip and went ahead to elongate as normal roots (Fig. 2 A).

The effective concentrations for inducing roots ranged for the six substances from 0.1 per cent to 1.0 per cent when dissolved in lanolin and applied along the region of elongation. Alpha-naphthaleneacetic acid was toxic at 1.0 per cent, but was very effective at 0.05 to 0.5 per cent. Phenylacetic acid, indolepropionic, and indolevaleric acid were only slightly active at 0.2 per cent but were very effective when 1.0 per cent was used. Only one application was necessary for inducing roots if the concentration was 0.2 per cent or more of naphthaleneacetic acid, indolebutyric acid, or indoleacetic acid. Higher concentrations—approximately 0.5 per cent—were necessary for the other substances. The time necessary for resumption of growth at the tip varied with the concentration, the quantity of the preparation used and the nearness of application to the point of the root. If 1.0 per cent indolebutyric acid was applied over the region of elongation even with, but not over the tip, growth was resumed within six to eight days. Recovery came earlier with indoleacetic than naphthaleneacetic acid. Elongation was greatly retarded, but not stopped, with 0.1 per cent of these substances. When roots were initiated with low

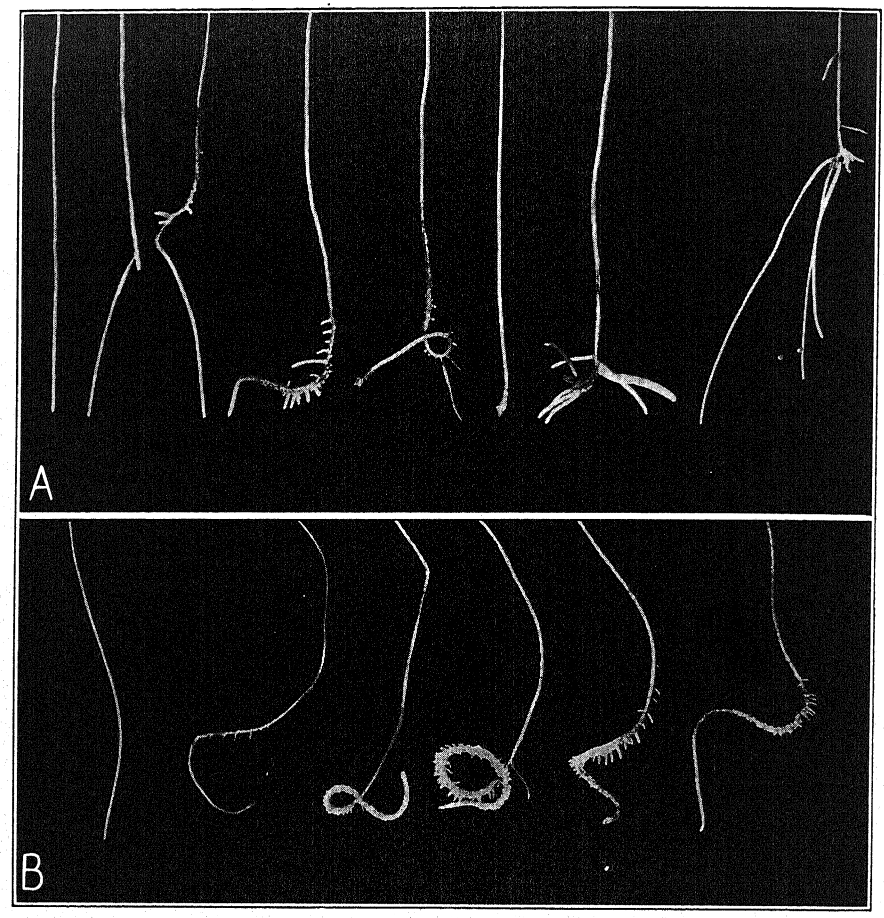

FIGURE 2. Response of aerial roots of *Vitis* sp. to root-forming substances. (A) Treatments with lanolin preparations of naphthaleneacetic acid. Left to right: (1) control; (2) response to excised tip; (3) treated with 0.1 per cent over region of elongation; tip recovered, retarding new roots; (4) treated with 0.5 of 1 per cent; tip beginning to recover; (5) treated over region of elongation with 0.5 of 1 per cent and after slight recovery, tip was re-treated, thereby preventing apical dominance; (6) treated with 0.5 of 1 per cent at tip only, causing swelling; (7) initiation and growth of roots after tip only was treated and re-treated to prevent recovery; (8) growth of roots after tip had been treated, then re-treated, thereby releasing 3 young roots which now dominate other roots. Photograph taken 12 days after first treatment. (B) Effect of immersing roots in solutions of naphthaleneacetic acid. Left to right: (1) control in air; (2) control in tap water; (3), (4), (5), and (6) in water solutions of naphthaleneacetic acid, 0.0006, 0.0003, 0.00015, 0.00007 per cent respectively. Photographed after 6 days in solution.

concentrations they made only slight elongation before being inhibited as a result of growth of the main root. When immersed in water over these retarded roots, the influence from the growing tip disappeared. Also, if the region producing the new roots was immersed in water, the tip remaining out of the water, there was no apparent inhibiting influence.

A lanolin preparation containing 0.5 per cent of the growth substances smeared over the tip of a root caused considerable swelling and, within five days, initiation of roots just back of the treated part. As with the other treatments the new roots were inhibited as soon as the main root renewed its growth. With one or two re-treatments, however, the new roots grew enough to be freed from the influence of the main tip (Fig. 2 A).

When many roots were initiated over a two-inch zone and then the tip injured or cut off, not all of the new roots continued to grow. There appeared to be even competition for a short time, then two or three roots gained an advantage and thereafter all others ceased to elongate (Fig. 2 A).

Application of the growth substances back of the region of elongation was comparatively ineffective. This might have been due to an impervious layer of collenchyma such as described by Turner (4) for roots of *Vitis rotundifolia*. He believes those roots to be similar to the ones of the unnamed species used in these experiments. When the surface of the root was scraped with a knife and the substance applied to the injured area a few roots were initiated in the adjacent regions. This type of response supports the conclusions of Turner.

When the ends of roots were immersed in solutions of growth substances, an abnormal thickening and a large number of new roots occurred, as compared with similar roots in tap water. Though many new roots were initiated in a solution of 0.0006 per cent naphthaleneacetic acid, very little growth was made. As the concentration was reduced there was less retardation as shown in Figure 2 B. A concentration of 0.00007 per cent initiated many more roots than the water control and there was practically no retardation. Roots which were induced in the water solutions of growth substances grew in rows along the region of elongation. The indications were that the cells forming the roots were associated with longitudinal strands of tissue.

As mentioned earlier the grape vines normally produce aerial roots from the nodes at frequent intervals. Seldom more than one root per node was found and never were roots produced on internodes of normal vines. The same substances which initiated roots on aerial roots were also effective on the stems. When applied at nodes or internodes many roots arose from the treated region. The substances were most effective on portions of the vine that were within the region of elongation. Roots were best induced four to six nodes back of the growing tip. In this respect the response of vines differs from that of roots. Nevertheless, the same com-

pounds induced roots on both stems and roots. They must therefore be true root-inducing substances.

There are two points of special interest in the results presented in this paper: first, that growth substances induce new roots to form on roots; second, that the growing tip exhibits a marked dominance over newly initiated branch roots but loses this power when it is artificially retarded with synthetic growth substances or immersed in water. In contrast with the latter response, when stem tips were treated with growth substances the lateral buds were inhibited (2).

Since all of the chemical compounds mentioned in connection with these experiments have the capacity to induce roots to form on stems, leaves, and on roots themselves, it seems reasonable to call such agents "root-forming substances." As to whether they act directly or indirectly is of little concern at this time. The evidence at hand seems to favor direct action. When the tip only is treated, new roots form immediately back of this point; treated along the region of elongation, the roots form only there; if a zone eight inches back of the tip was treated, elongation was retarded and roots formed all the way down. The indications were that the substances moved more readily toward the tip than otherwise. However, elongation was greatly retarded when only the tip was treated.

It would be of interest to know exactly why the aerial roots of *Vitis* do not branch until they strike soil, or water, or are given special treatment with chemical compounds. This seems to point to a very strong apical dominance which may mean the production of a substance by the growing tip, exerting an influence back along the root. If so, such agents of plants resemble the chemical messengers of animals commonly called "hormones."

It is significant that the tip must be in an active state of growth to dominate branch roots. In fact, these branch roots can grow only when the tip is retarded or interfered with by some means.

A question arises as to what becomes of the powerful dominating agent when the growing part of the root is immersed in water. If the factor is a chemical substance it might, of course, leach out of the tissue into the water. To do so, however, calls for a change in direction of movement. Another view might be that under such conditions the root absorbs water and so dilutes the dominating substance that it is no longer effective. While growing in air all food materials and water must be sent to the tip from the vine. When the root reaches the soil it takes up a new function, that of supplying water and mineral elements to the vine. The root, therefore, assumes its true function and there must begin an active flow of materials through the xylem. It is conceivable, therefore, that any special materials manufactured at the growing tip could get into the transpiration stream and be carried back to the vine.

Another theory, that of competition for food, was suggested as a possible cause for failure of some branch roots to grow. That does not seem possible for two reasons. First, roots nearest the source of food supply are often the ones most retarded; second, when the root is immersed in water all roots grow, though the same competition should maintain.

SUMMARY

1. An unnamed species of tropical grape (*Vitis* sp.) growing in a greenhouse produced aerial roots which did not branch until coming in contact with soil or other moist material. If the root was severed, one or two new roots arose just above the cut surface. The roots normally elongated an average of four inches per day.

2. A study was made of the root-forming power of six chemical growth substances when applied to roots. The substances, α-naphthaleneacetic acid, indolebutyric acid, indoleacetic acid, indolepropionic acid, Δ-(3-indolyl)-valeric acid, and phenylacetic acid, were used as lanolin preparations and as water solutions.

3. When lanolin preparations of the substances were applied along the region of elongation new branch roots appeared through the epidermis in three to five days. The substances also caused swelling and retardation in elongation of the roots.

4. The growing tip was found to have a dominating influence over branch roots. The newly initiated roots were inhibited when the tip of the main root resumed growth after having been retarded by the substance applied to it. By re-treatment of the tip the new branch roots could be induced to grow. Also, if the region producing the new roots were immersed in water the apical dominance was overcome.

5. Application of the growth substances back of the region of elongation was comparatively ineffective. Either the material did not penetrate or old tissue is not as susceptible as that of the growing region.

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ABSORPTION AND MOVEMENT OF SYNTHETIC GROWTH SUBSTANCES FROM SOIL AS INDICATED BY THE RESPONSES OF AERIAL PARTS

A. E. Hitchcock and P. W. Zimmerman

Since relatively small amounts of growth substances induce specific types of formative responses on plants (6), these responses may be used as a means of detecting the presence of the compounds in certain parts of the plant. It has already been shown that ethylene (17) and a number of the crystalline growth substances (6, 8, 18) move readily through the plant, but it has not been established through what channels and in what manner these substances are transported. The fact that pronounced bending responses occur in one hour or less after treatment, means that the rate and direction of movement of the growth substance may be determined within a relatively short period of time. It is thus possible with this method to subject the test plants to a wider range of atmospheric conditions than would be feasible for methods which require several days or longer.

Earlier reports have dealt with substances applied to aerial portions of plants. It now appears that plants can absorb synthetic growth substances from the soil as they do mineral elements. The absorption of growth substances from the soil was influenced by the rate of transpiration. Transpiration also influenced the rate of movement in aerial parts when the growth substances were introduced as water solutions in the transpiration stream, but not in the case of lanolin preparations. The efficiency of the absorption system for the intake of growth substances from the soil is believed to be essentially no different from that whereby the common mineral elements enter the plant. Failure of previous investigators to establish any relationship between the absorption of mineral substances and the rate of transpiration is no doubt due to the improper selection of the experimental conditions. As has been pointed out by at least one investigator the minimum conditions for absorption of the mineral elements have probably not been reached in tests which appear to show no influence of transpiration on absorption (7, p. 75).

The addition of growth substances to the soil not only induced all of the responses obtained by applying the material to the aerial part of the plant, but it proved to be the most effective method for causing systemic bending and rooting responses on tomato and tobacco plants. The premature flowering on Turkish tobacco which resulted from the soil treatments is a response not previously reported for the particular compounds used. Similar effects, however, have been reported for the follicular hormones on hyacinth (12). The influence of light on stem bending is also an

effect not previously mentioned. In fact, this response constituted the most sensitive test for determining the presence of the growth substance in the upper part of the stem.

The fact that lanolin preparations of the growth substance failed to induce systemic responses on tobacco (8, 9), whereas the soil treatments did, would appear to be a matter of the growth substance reaching the longitudinal conducting channels in the one case and not in the other. Living tissue was not required for the transport of the growth substances, and there was no evidence of a polar movement such as has been reported for auxin A in *Avena* coleoptiles (3, 4, 5, 14, 15). These results indicate that when the growth substances are introduced as water solutions, they move longitudinally from the point of application to the region of response in the xylem. The present paper deals particularly with the absorption and transport of the synthetic growth substances added to the soil and with the possible bearing which these results may have on the absorption and transport of materials in general. A detailed description is also given of the principal responses induced by the soil treatment method.

MATERIALS AND METHODS

Crystalline compounds. The following six compounds were used: indoleacetic, indolebutyric, indolepropionic, naphthaleneacetic, phenylacetic, and phenylpropionic acids. The indole compounds were obtained from Dr. R. H. Manske and naphthaleneacetic acid was prepared in this laboratory according to the method previously cited (18, p. 210). The phenyl compounds were obtained from the Eastman Kodak Company. Water solutions of these compounds were prepared by first dissolving the crystals in a few drops of 95 per cent ethyl alcohol and then making up to volume with tap water (generally, either 40 or 50 cc.). The solution containing the growth substance was poured on the surface of the soil. All plants were grown in 4-inch pots which held approximately 450 g. of air-dry soil. A few comparative tests were also made with lanolin preparations of the compounds. In this case the substance was applied to the aerial part of the plant. Root systems of young tomato seedlings were washed free of soil and then placed in tap water containing one of the compounds in order to determine the minimum period of absorption necessary to induce a bending response on the aerial parts.

Killing stem tissue. Portions of tomato and tobacco stems were killed by wrapping flexible resistance wire in a spiral around the stem and then passing an electric current through the wire. The length of the segment killed varied from one-half to two inches. A uniformly collapsed strand resulted from this method of killing. After several hours, the dead tomato tissue had shriveled to a diameter of 1 to 3 mm. No means were used to prevent the shriveled strand from drying.

Solution of dye. Fast Green dye was used to determine whether or not the root systems of young tomato seedlings were intact. These particular seedlings had been grown in nutrient solutions for two weeks. After responses to indole compounds were obtained, some of the dye was added to the solution surrounding the root system in order to determine whether the indole compound had passed through intact membranes. Failure of the plant to take up the dye was regarded as an indication of an intact root system. Seedlings with injured root systems took up the dye readily. The dye was also admitted to cut surfaces of stem and leaves in order to compare the transport of this substance with that of the synthetic organic acids. Exact rates of movement were not determined.

Plants used. Tomato (*Lycopersicon esculentum* Mill. vars. Bonny Best and Marglobe) and tobacco (*Nicotiana tabacum* L. var. Turkish) were the principal test plants. A few tests were made with the African marigold (*Tagetes erecta* L.). Tomato plants were from 1 to 26 inches in height. Tobacco plants ranged from 6 to 30 inches in height at the time of treatment. The plants were grown in 4-inch pots which held approximately 450 g. of air-dry soil.

EXPERIMENTAL RESULTS

PLANT RESPONSES WHICH INDICATE THE PRESENCE OF THE GROWTH SUBSTANCE

Growth substances are detected and the relative amount measured by the physiological responses they induce. In the case of auxin A the bending response on *Avena* coleoptiles has been the principal means of determining the relative amounts of this substance in different tissues, the rate of movement, and something concerning the polarity of the movement. The bending response on coleoptiles is a growth response involving cell elongation and the curvature is caused by a greater elongation on one side than on the other. A curvature of 10° is induced on the coleoptiles by a 2 cubic mm. block of agar containing 20×10^{-6} to 90×10^{-6} mg. of auxin A (3, p. 24). Considering the average activity as 55×10^{6} AE, this value is equivalent to 1 part of auxin to 110,000,000[1] parts of water. The epi-

[1] The effectiveness of 1 part of auxin to 50,000,000,000 parts of water originally assigned to auxin A (6, p. 239) was incorrectly based on the assumption that both solute and solvent were on the same (gram) weight basis. Boysen Jensen (3, p. 24–25) has pointed out the confusion which has resulted from the use of various auxin units ranging in value from 1 to 400,000. It is unfortunate that a simple basis such as parts per million was not chosen, or that when referring to the effectiveness of a given weight of auxin, this weight was not stated in milligrams instead of grams. The corrected (average) value of 1:110,000,000 given in the present paper for auxin A indicates that this auxin is from 1000 to 6000 times less effective than ethylene which is active in a concentration of 1:658,000,000,000 on a tissue weight basis. Even on the molecular equivalent basis ethylene is about 100 to 600 times as effective as auxin.

nastic response of tomato leaves is in many respects similar to the bending response of *Avena* coleoptiles. This and other similarities between the responses induced on *Avena* by auxin A and those induced on the tomato and several other plants by a number of synthetically prepared chemical compounds including hetero-auxin (indoleacetic acid) have been discussed in an earlier report (6).

The responses induced by adding the growth substances to the soil were qualitatively the same as those resulting from treatment of the aerial part of the plant with lanolin preparations or solutions of the same substances. Since the movement of a particular growth substance from one part of the plant to another was determined by the formative responses induced there, the success of this method depends upon a thorough knowledge of the various types of responses which these synthetic compounds are capable of causing on the test plant used. Consequently, a detailed description of the principal responses is given in this section of the paper.

Leaf bending. An epinastic response is a growth response resulting from a treatment which induces a faster rate of growth on the upper side of the leaf than on the lower side. This region of response may be limited to one part of the leaf or it may include nearly all parts, depending particularly upon the species of plant and the age of the leaf. While in general an epinastic response means downward bending, in some cases it consists also of curling, coiling, or twisting.

Treatment of the soil with any one of the six compounds induced epinasty of some or all leaves of tomato plants ranging in height from 1 to 26 inches. The upper leaves usually showed the first signs of bending, followed in order by the middle and lower leaves. Downward curling of the tip leaflet constituted the first stage of the epinastic response of a single leaf. This was followed by curvature of the midrib and a declination of the petiole. Under optimum conditions epinasty became evident in one hour or less. Pronounced responses such as are shown in Figure 1 A occurred at the end of two hours.

The degree and rate of bending depended upon the age and activity of the test plant and of any particular leaf, the kind and amount of compound used, and the atmospheric conditions. Three milligrams per 450 g. of soil in the case of all compounds except phenylpropionic acid caused epinasty of most leaves on actively growing plants four to six inches in height. In order to induce a similar response on larger and older plants it was generally necessary to use larger amounts of these compounds (10 to 20 mg.). Relatively slow growing plants with hard stems were much less sensitive than plants of the same age which had been maintained in an active state of growth and hence were composed of more succulent tissue. Thus the type of growth appeared to be of more importance than the age or height of the plant in determining the sensitivity and magnitude of the

epinastic response. Increasing the amount of the compound resulted in a more pronounced epinasty, but an amount was finally reached which delayed the initial response.

Indole compounds were more effective than phenyl compounds in causing epinasty, particularly in the case of plants ten inches or more in

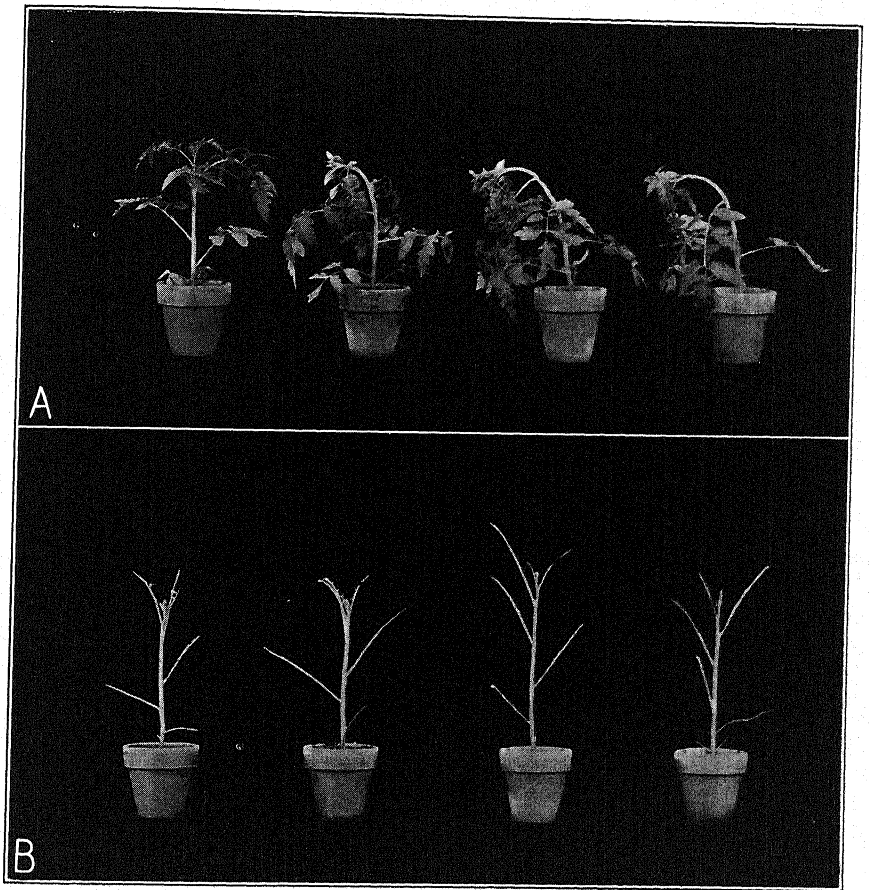

FIGURE 1. Effect of removing leaflets on the response of tomato plants two hours after adding crystalline growth substances to the soil. Ten milligrams of the compound dissolved in 50 cc. of tap water were added as follows, from left to right: none (control), indoleacetic acid, indolebutyric acid, and indolepropionic acid. A. Intact plants. B. Plants with leaflets removed.

height. In the case of minimum active amounts, indoleacetic acid was more effective than the other two indole compounds, but the differences were not nearly so marked as has been reported for lanolin preparations applied to the aerial part of the plant (6, 8, 18). The use of more than 20 mg. of

the indole compounds injured or killed the underground parts of the plant, but before this type of injury became evident, the plant showed a typical epinastic response. Amounts in excess of 50 mg. of the phenyl compounds were likewise injurious.

Recovery of leaves to their original position depended upon the kind and amount of compound used, age of the plant, age of the leaf, and the atmospheric conditions, particularly with reference to light. In the case of minimum active doses (approximately 3 milligrams) nearly complete recovery of leaves occurred in 24 hours. Plants treated with more than 3 mg. of indoleacetic acid showed better recovery than those treated with equivalent amounts of the other two indole compounds or with the phenyl compounds. There was little or no recovery, for example, when 9 to 15 milligrams of the indole compounds were used. In contrast to these results there was complete recovery of plants receiving an application of 27 milligrams of phenylacetic acid. When a water solution of the growth substance was introduced through the cut surface of a tomato petiole and the plant was then placed in the dark, most leaves showed pronounced bending in six hours at which time the vial containing the solution of growth substance was removed. At the end of 48 hours nearly complete recovery of the leaves had occurred in darkness. In contrast, there was little or no recovery of leaves in darkness in the case of plants treated with lanolin preparations of the growth substance. Failure of the leaves to recover on plants treated with lanolin preparations was apparently not due to the inability of the leaves to grow in darkness during the first 48 hours.

Atmospheric conditions proved to be the principal limiting factor in determining the rate and degree of leaf bending, regardless of the amount of compound used or the age and activity of the test plant. The details of these results are given in a separate section of this paper which deals with conditions affecting the absorption and transport of growth substances.

Although what has been described for the tomato applies in the main also to tobacco plants, certain exceptions were noted. Epinasty of tobacco leaves was much slower (a noticeable response not being evident until from 3 to 6 hours or longer) and the final response was usually less pronounced than on the tomato. The upper three leaves on tobacco plants never showed a noticeable epinastic response. On the contrary, these leaves which ranged from one and one-half to four inches in length exhibited hyponasty (upward bending), particularly in the case of effective treatments that induced pronounced stem bending. These responses occurred on plants 10 to 12 inches in height and in an active state of growth. The three to five leaves below the ones showing hyponasty gave the most pronounced epinastic response. Injection tests also showed that the same solution induced hyponasty on the upper leaves and epinasty on the leaves below. Although upward bending is the position assumed

by these leaves at night, the injected solution of indolebutyric acid induced an arched upward bending of the midrib which is not characteristic of the "night" position. Furthermore, inward rolling of the margins also occurred on these same leaves. Similar hyponastic responses have been reported for plants exposed to illuminating gas (10, p. 174). Indolebutyric acid was much more effective than indolepropionic, phenylacetic, or phenylpropionic acids. In contrast to the systemic bending response on tobacco resulting from soil treatment, the application of lanolin preparations to the petioles or stems causes local responses only (8, 9).

Stem bending. The degree and rate of stem bending and of recovery were influenced by the same factors and to approximately the same extent as has been described for epinasty of leaves. Under certain conditions, particularly with small amounts of the compound (1 to 8 milligrams per pot), stem bending was more pronounced than leaf bending one hour after treatment. Bending occurred at various places from slightly below the stem tip to within one-half inch of the soil (Figs. 1 A and 2 A). Ten milligrams or more of the indole compounds generally induced a permanent bend on stems of young tomato plants. Definite stem bending occurred in from 30 to 40 minutes, and it was pronounced at the end of one hour. The initial bending usually occurred at or near the tip and was independent of stem height in the range from 2 to 26 inches for actively growing plants. Generally, however, plants 6 to 9 inches in height responded more rapidly than shorter or taller plants, but the difference in time was not proportional to the height of the stem. For example, initial bending started 50 to 70 minutes after treatment on plants 2, 5, 16, and 26 inches tall. The tip leaf at the growing point (one-half to one inch in length) bent upward and over in the same direction and simultaneously with the stem. This particular leaf showed a hyponastic response.

Initial bending was usually toward the light. Bending toward the light was most pronounced in the laboratory where the windows were on one side of the room. In the greenhouse stem bending toward the light was also noticeable and was in opposite directions for plants on top of the bench and the ones under the bench due to the fact that the main source of light on top of the bench was from the south and under the bench from the north. Although stems of the control plants under the bench in the greenhouse and those in the laboratory eventually bent toward the light, the response was much slower than on the treated plants and the type of bend was distinctly arched and not sharp. When the solution of growth substance was introduced through the cut surface of a leaf, the stem first bent away from the treated leaf and later toward the treated leaf. This response occurred on plants in light and also on those in darkness, so that in this case the direction of stem bending appeared to be correlated with the point of entry of the growth substance and not with the source of light.

FIGURE 2. Absorption and transport of growth substances added to the soil as indicated by the responses on tomato. A. Left, plant kept under bell jar in laboratory for 18 hours after 5 mg. indolepropionic acid had been added to the soil. Right, plant given similar treatment but remained in the open. B. Left, control plant with dead stem segment. Right, similar plant five hours after 8 mg. of indolepropionic acid had been added to the soil.

FIGURE 3. Rooting on Turkish tobacco 20 days after 27 mg. of indolebutyric acid had has been added to the soil. Permanent stem and leaf bending are also shown.

Stem bending on tobacco was less pronounced than on the tomato (Fig. 3), the relative difference being about the same as was described for epinasty of leaves. Indolebutyric acid was more effective than indolepropionic, phenylacetic, or phenylpropionic acids. Bending of the upper part of tobacco stems was also toward the main source of light as in the case of the tomato, but the direction of bends on the lower part of tobacco stems was irrespective of light source.

Types of proliferation on stems. Treatments which caused marked stem bending also induced swelling. Optimum treatments caused noticeable swelling on all parts of the stem except at the tip and also at the base of the petioles. Indole compounds were much more effective than the phenyl compounds and indolepropionic and indolebutyric acids were the most effective of the six compounds used.

Other types of proliferation were obtained with soil treatment which were identical with the responses previously described as being induced by the application of these same substances to the aerial part of the plant (8, 18). Small amounts of the compounds caused the formation of small scattered patches that eventually became light green in color or nearly white. Frequently these patches were located in rows along one or more of the vertical ridges on the stem. Increasing the amount of the compound applied, caused a corresponding increase in the number and the size of these patches until practically the entire stem became light green or nearly white in appearance. Proliferations also appeared on petioles and midribs of leaves. Stem swelling and change in color were noticeable at the end of 24 hours. Naphthaleneacetic acid induced a much whiter and a more fluffy type of proliferation than the other compounds. Large amounts of the indole compounds caused strips of the outer stem to peel off. The phenyl compounds did not cause this type of response.

Proliferations on tobacco were similar to those on the tomato, and in the case of plants 10 to 12 inches in height the proliferations extended to within one inch of the tip. On older and taller plants there was little or no proliferation. Indolebutyric acid was more effective than indolepropionic, phenylacetic, or phenylpropionic acids.

Initiation and stimulation of roots. Root growth from the stem of tomato was essentially the same as has been described for the application of these compounds to the aerial part of the plant (8, 18). This applies to the time for roots to appear, the portion of the stem from which roots emerge, the total number of roots (several hundred, at least in the case of optimum treatments), and the differences due to the kind and relative amount of the substance used. For example, treatment of the soil with naphthaleneacetic acid induced roots which grew vertically downward after emerging from the stem and soon became covered with a profuse growth of root hairs. Similar treatment with phenylacetic acid caused

FIGURE 4. Rooting responses on tomato induced by adding growth substances to the soil. A. Left, control; right, 10 mg. naphthaleneacetic acid (roots grow vertically downward and are covered with a profuse growth of root hairs). B. Left, control; right, 200 mg. phenylpropionic acid (roots grow out at an angle of about 45° and show sparse root hair growth). Photographed 11 days after treatment. Note also retarded growth.

roots to grow downward at approximately an angle of 45 degrees and there was a relatively small amount of root hair growth. These differences are illustrated in Figure 4. In relatively large amounts (particularly in excess of 20 milligrams) indole compounds induced roots which resembled those resulting from treatment with minimum active amounts of naphthaleneacetic acid. This was particularly true for indoleacetic acid.

Roots induced by optimum amounts of phenylpropionic acid appeared sooner and in larger numbers during the first eight days than roots induced by the other compounds. Phenylpropionic acid was the only compound which induced many roots to appear from parts of the stem that did not exhibit noticeable swelling or other types of proliferation (Fig. 4 B). The appearance of roots from the lower part of the stem three to four days after treatment of the soil with phenylpropionic acid indicated that this compound stimulated the growth of root primordia as well as induced the formation of new primordia. Phenylacetic acid was the only other compound which induced a similar reponse. Large amounts of the indole and naphthalene compounds induced the formation of roots which were abnormal with respect to diameter, rate of growth, root hair growth, and color, as previously described (18). Some of these roots were devoid of root hairs.

Indole and naphthalene compounds were more effective than the phenyl compounds not only from the standpoint of the minimum amounts required to induce rooting, but also because proportionally larger amounts could be used before the toxic limit was reached. Naphthaleneacetic acid was the most toxic to root systems in soil. The quantity of the compound which induced optimum rooting was usually less than ten times the amount which caused minimum rooting. This range depended mainly upon the age and activity of the test plant. Amounts much in excess of the optimum generally induced abnormal roots.

In addition to root growth from the stem of the tomato, roots also appeared from the petiole and along the entire midrib of the middle leaves. This type of response was induced by indolepropionic acid (27 milligrams per pot). Roots induced by naphthaleneacetic acid were of a different type on tobacco and marigold than those on tomato. Roots on the tobacco grew downward at an angle of approximately 45° but they were not covered with a profuse growth of root hairs. In the case of marigold, roots grew out horizontally or slightly declined from this position. On both of these plants the roots were whiter than those induced by naphthaleneacetic acid on the tomato.

Indolebutyric acid was especially effective in causing roots to be initiated in stems of tobacco plants 10 to 12 inches in height. Optimum rooting resulted from the addition of 27 milligrams to the soil (Fig. 3). Noticeable swelling of the lower eight inches of the stem was evident after two days.

At the end of six days longitudinal cracks appeared in many places in the epidermis from the soil line upwards for a distance of five inches. Eight days after treatment the cracks widened sufficiently to expose masses of young roots underneath, and new cracks continued to appear. At the end of 12 days it could be seen that a solid mass (at least several thousand!) of roots was emerging from the lower eight inches of the stem. The roots were so close together that stem tissue was not visible between them anywhere along this eight-inch segment. Many roots were emerging from the next two inches above, but they were somewhat scattered at first, although later showing a solid mass as in the eight-inch segment below.

Early flowering. Flowering of the Turkish tobacco was hastened by application of the growth substances to the soil. The greatest differences were obtained when the plants were treated three to five weeks prior to the time the control plants flowered. In one experiment three different concentrations of each of four different growth substances were used on plants 10 to 12 inches in height. Each treatment was replicated three times. In the case of indolebutyric, indolepropionic, and phenylacetic acids the following amounts were used: 3, 9, and 27 mg. The amounts of phenylpropionic acid added to each pot were 9, 27, and 54 mg. At the end of three weeks flower buds were visible on 16 of the 36 treated plants and on 1 of the 12 control plants. The average height of these plants at the time of treatment was 11.7 and 11.0 inches respectively, and three weeks later 22.3 and 20.7 inches respectively. Treated plants with flower buds were therefore not retarded by the amounts of growth substance used in these tests. The average height of the 20 treated plants which did not have flower buds was 19.6 inches. This slightly lower average value was due to the marked retardation of growth caused by one of the indolebutyric acid treatments which consisted of an application of 27 mg. The two highest doses were most effective in hastening flowering, except in the case of indolebutyric acid. Although this amount of indolebutyric acid was not effective in hastening flowering, it proved to be the optimum amount for inducing roots. The two smaller doses (3 and 9 mg.) of indolebutyric acid hastened flowering, but they were much less effective than the larger dose (27 mg.) in causing root formation. Three other experiments in which phenylacetic acid was used gave similar results.

Retardation of growth. Any of the treatments which induced permanent bending, proliferation, or rooting also retarded stem elongation. The degree of retardation depended principally upon the kind and the amount of the growth substances used. The marked retardation shown in Figure 4 resulted from treatment with 10 to 20 milligrams of naphthalene or indole compounds. Tobacco and marigold were retarded in a similar manner. The minimum dosages for bending responses on the tomato caused no appreciable retardation.

CONDITIONS AFFECTING ABSORPTION AND MOVEMENT OF GROWTH SUBSTANCES

Influence of atmospheric conditions. It was observed that the same treatments were less effective on cloudy days than on bright days. Subsequent tests showed that the initial bending responses occurred most rapidly and the final responses were most pronounced under conditions which caused a high rate of water loss from the plant. Bending responses were delayed or prevented by placing the plants under bell jars in the laboratory (Fig. 2 A). In the case of minimum active amounts of one of the six compounds there was no noticeable bending response on the tomato plants under bell jars, even during a period of three days. The use of slightly larger amounts of the compound induced bending on the plants under the bell jars either in light or in darkness after from three to five hours or longer. Similarly treated plants that remained outside the bell jars and in light exhibited marked bending responses in one and one-half to two hours. The final response of the plants which had remained under bell jars for several hours and were then transferred to the greenhouse was always much less pronounced than the response of plants which had remained outside of the bell jars. The temperature under bell jars in bright light was usually 2° to 3° C. higher than the temperature of the air where plants were in the open in light or in darkness.

The bending responses were delayed for a longer period and the final response was much less pronounced if the bell jars were placed in the dark. Light, however, was not the principal limiting factor in affecting these responses. Although plants placed in reduced light or in darkness responded more slowly than plants in good light, the former exhibited a marked response before the plants under bell jars either in light or in darkness had responded. That the principal limiting factor was directly related to water movement in the plant and not to light was further shown by tests in which the plants were placed in darkness immediately after treatment for periods of 10, 20, and 30 minutes and were then removed to full light in the greenhouse. The plants which had been under bell jars showed a more pronounced initial response (after one and one-half hours) and final response (after 18 hours) than the plants remaining outside of the bell jars and in darkness. In this case the increased transpiration induced by a change from a high to a lower humidity presumably caused an increased absorption of the compound which had been added to the soil.

Certain additional tests showed that in from 10 to 30 minutes a sufficient amount of the compound was absorbed by the plants under conditions of high evaporation to induce pronounced bending responses under conditions of low evaporation. Plants remaining on top of the bench in the greenhouse for periods of 5, 10, 20, and 40 minutes after treatment were then transferred to bell jars under the bench. Forty minutes after treatment bending responses were not evident on any of these plants. At the

end of 90 minutes pronounced bending of stem and leaves had occurred on treated plants which had remained on top of the bench continuously and on the plants that had remained on top of the bench for 20 or 40 minutes after treatment. The plants placed in bell jars under the bench at the time of treatment and five or ten minutes after treatment showed no response at this time, or at the end of three hours. After 18 hours (overnight) all plants showed a bending response of stem and leaves, the degree of bending being proportional to the time the plants had remained on top of the bench before removal to the bell jars. There was slightly more bending on the ten-minute plants than on the five-minute plants. Stems of the 40-minute plants had been to the horizontal position and were identical in appearance with the ones remaining on top of the bench. In these particular tests nine milligrams of indolepropionic acid were added to the soil of each test plant. This amount is from three to nine times the minimum quantity that will induce marked bending responses.

That a relatively rapid rate of absorption of these special compounds occurs, was further demonstrated by tests in which the root systems of young tomato seedlings were placed in the test solutions for different periods of time and were then washed and transferred to tap water. The roots were able to absorb enough indolepropionic acid in five minutes to induce a marked bending response in two hours. The minimum time period was not determined. Bending responses for the different time periods were directly correlated with the concentration of the solution. In the case of 240 milligrams per liter the response on plants left in for five minutes was equivalent to that on seedlings left 30 minutes in a solution containing 80 milligrams per liter. Absorption in this case was no doubt mainly through broken or injured parts of the roots. However, tests with intact root systems of tomato seedlings grown for two weeks in nutrient solutions showed that the growth substances passed through membranes which excluded Fast Green dye. Injured root systems absorbed the dye readily. Likewise, injured root systems absorbed the growth substance much more readily than an intact root system.

Stem bending on treated tomato plants was a more sensitive response than epinasty of leaves when the plants were subjected to a distinctly one-sided illumination such as was furnished by the conditions under a side bench in the greenhouse, on top of the laboratory bench in a room where there were windows on only one side, and on top of the greenhouse bench (in the fall when the angle of the sun is low). Leaves also turned toward the source of light under these conditions, but this type of movement was not so readily detected as the declination of the stem. Stems of treated plants under bell jars in the laboratory responded more slowly or not at all, depending upon the amount of the chemical compound which had been added to the soil.

The response of the tobacco to different atmospheric conditions was

essentially the same as was described for the tomato. Stems of treated tobacco plants 10 to 12 inches in height bent toward the main source of light. Only the upper part of the stem responded in this manner. Bends lower down the stem which were also the result of soil treatment were not correlated with the main source of light. Tobacco plants showing this type of stem bending were located on the greenhouse bench. Treatment was given at 11:30 a.m. and the responses observed between 4:00 and 5:00 p.m. Stems which had bent toward the main source of light at this time (south to southwest) recovered to the original vertical position during the next 24 hours. Bends farther down the stem were permanent. Hyponasty of one or more of the upper three leaves (one and one-half to four inches in length) appeared not to be correlated with the main source of light since leaves on both sides of the plant showed the response and hence were bending in opposite directions. Furthermore, hyponasty was still evident after two days and at a time when complete recovery of the upper stem had taken place. Stem bending, hyponasty, and epinasty of leaves were induced on a single plant by the same treatments.

Proliferation and rooting responses were also affected by atmospheric conditions. For example, the tomato plants removed from the bell jars five hours after soil treatment showed only slight proliferation and no rooting at the end of ten days, whereas the plants which had been outside the bell jars showed marked proliferation and many roots had grown from the lower four inches of the stem.

The response of tomato plants to lanolin preparations of the same compounds was not influenced to the same extent by atmospheric conditions as the responses of plants given soil treatment. In the case of lanolin treatments during the latter part of September, the response of tomato plants located on top of the bench, under the bench, in the laboratory under bell jars, and outside either in light or in darkness was approximately the same. Any delay in responses was generally on plants subjected to the highest light intensity. During the middle of the day and on bright days this delay was on the plants on top of the greenhouse bench. Treatments after 3:00 p.m. were not appreciably influenced by atmospheric conditions. These results show that the absorption of the substances causing bending, proliferation, or rooting responses was different in the case of lanolin applications than when the same substance was added to the soil.

The fact that lanolin preparations did not induce a systemic response on tobacco plants, when applied to the petiole, indicates that the compound failed to get into the same channels of transport as occurred in the case of soil treatments. Systemic responses resulting from treatment with lanolin preparations varied according to the age of the tissue treated. For treatments on lower portions of the tomato stems the initial response was

much slower and the final response was much less pronounced than when the preparations were applied to younger tissue. Recovery of the plants in either case was more rapid and more complete in full light than in reduced light or in darkness.

Removal of leaflets. Removal of all leaflets from the midribs of tomato leaves delayed bending responses on stem and petioles. When minimum active amounts of the compound were added to the soil, no bending occurred (Fig. 1 B). Larger amounts of the compound induced curvatures on petioles, midribs, and stem, particularly on young actively growing plants four to six inches in height, but the responses were slower than on similarly treated intact plants. There was little or no recovery of stem and petioles, whereas the intact plants made complete recovery. With plants 10 to 20 inches in height much larger amounts of the chemical compound were required to induce bending of the petioles as compared to the requirements for plants four to eight inches in height. That the stem and midribs of these plants had the capacity to bend was shown by the fact that bending was readily induced by lanolin preparations of growth substances.

Transport through dead stem tissue. The substances which induced bending of stem and leaves moved both upward and downward through a segment of dead stem tissue from one-half to two inches in length. Responses such as the one shown in Figure 2 B resulting from soil treatment were also obtained by applying a lanolin preparation of the growth substance to a portion of the stem just below the dead segment. Success with lanolin preparations depended upon the succulence of the tissue treated. Upward transmission of the chemical through a dead segment by means of lanolin preparations was obtained only with soft stem tissue. Responses above the dead segment were also obtained by injecting a solution of the growth substance into the base of the first petiole below the dead segment. Although no attempt was made to determine exact rates of movement, downward transport through dead stem tissue appeared to be much slower than upward movement in the tomato and tobacco. The downward transport in this case resulted from admitting solutions of the growth substance to cut surfaces of stem or leaves, but not as a result of lanolin treatments. Both in the case of intact stems and those containing a dead segment, responses at distant points were more readily obtained with solutions of the growth substance than with lanolin preparations. Longitudinal movement of the growth substance in either direction through the dead segment or through intact stems was retarded or prevented by placing the plants under bell jars in light or in darkness.

In from three to five days after killing the stem tissue, axillary buds below this region started to grow on the control plants in much the same manner as when the plant was decapitated (9, p. 357). Both tobacco and

tomato showed this response, but the differences on the former were more consistent due to the fact that axillary shoots do not develop until the plant flowers. These results show that substances produced in the tip of these plants were not transported through dead stem tissue, although passage of the synthetic growth substances was effected in both directions. When it was established by bending responses that the synthetic growth substances had passed through the dead segment, the growth of the axillary buds was noticeably retarded or inhibited. The synthetic growth substances therefore inhibit bud development the same as the substance in the plant which normally performs this function. If a segment of the central woody cylinder inside the bark was removed from tobacco stems, axillary buds grew from the portion of the stem below in the same manner as if the stem had been cut off. However, when a band of the bark was removed and the central woody cylinder left intact, the axillary buds did not grow. These results indicate that the substance in the plant which inhibits the growth of lateral buds does not move readily in a longitudinal direction through the bark tissue of the tobacco. Furthermore, the introduction of water solutions of growth substance through a slit portion of stem caused epinasty of one or more leaves above this point when the overhanging piece contained a sliver of the woody portion of the stem, but if only the bark dipped into the solution (the sliver of wood having been removed) no epinasty occurred. In this case there was no appreciable absorption of the solution by the bark tissue, whereas all of the solution in the vial (2.5 cc.) was taken up by the piece of stem containing both bark and wood. Thus water solutions of the synthetic growth substances did not move readily in a longitudinal direction through bark tissue.

Fast Green dye moved readily in both directions through the dead segment of a tobacco stem when the solution was introduced through the cut surface of a leaf. The path of transport was principally on the side where the dye was introduced. In a leaf eight inches below the dead segment the dye was visible in transmitted light in less than one hour after being introduced at a point eight inches above the dead segment. No attempt was made to determine the maximum rate of movement. Rapid upward movement of the dye through the dead tissue also occurred.

DISCUSSION

Similarities in the effects of ethylene and the plant auxins have been described in an earlier report (6). That the crystalline compounds used in this laboratory move as readily through the plant, and perhaps more readily than ethylene (17), has been demonstrated by the results reported in this paper as well as in previous reports (6, 8, 18). There are a number of differences, however, which indicate that explanations for the response of *Avena* will not apply to the response of the tomato, tobacco, or marigold.

Hetero-auxin, for example, is highly active in producing curvatures on *Avena* and on the tomato (6). In contrast, the closely related homologue, indolepropionic acid, is highly active on the tomato, but shows no activity on *Avena* (8). Furthermore, the transport of auxin A in *Avena* and in *Nicotiana* (1) appears to be entirely different from the transport of the synthetic compounds in tomato and *Nicotiana*.

It seems fairly well agreed that the movement of auxin A in the *Avena* coleoptile is strictly polar (3, p. 42; 14, p. 450; 15, p. 195). Avery (1, p. 322) has shown that the movement of auxin in leaves of *Nicotiana* is also polar. The movement of rhizocaline in cuttings of *Phaseolus* is likewise polar according to Went (16). There is lack of agreement concerning the polarity of auxin transport in roots (4, p. 349; 5, p. 529). In contrast to these results, there has been no indication in our experiments that the synthetic compounds, including hetero-auxin, show a strictly polar movement in growing plants. Likewise, ethylene gas moves in all directions through the plant (17). If the movement of auxin A is only downward in stems and leaves, then it must be assumed that the responses induced on the tomato by the synthetic compounds did not involve the upward transport of auxin A. Presumably the active principle which moved from the point of application to the region of response was the synthetic compound applied. In the case of soil treatments this movement was upward, and when the aerial part was treated the movement was both upward and downward.

The qualitative differences in root growth resulting from treatment with different synthetic compounds (18) is another reason for believing that the substance applied is the one which is transported and not a single plant auxin such as auxin A. Furthermore, the upward transport of the root-forming principle occurs not only in the stem of tomato, but also in the leaves. Compounds which are most effective in causing roots to be initiated are not necessarily the most active in causing the bending responses (13, 18). With soil treatments, naphthaleneacetic and indolebutyric acids were more effective than indoleacetic acid for the initiation of roots, yet the latter compound was the most active in causing epinasty of leaves. These results are in agreement with those previously reported for lanolin preparations of the same compounds (18).

The question of the channels of transport and the method by which substances are absorbed from the soil and carried to different parts of the plant are still matters of doubt and controversy (2, p. 64; 7). Thus any information concerning the absorption and transport of growth substances is not only important from the standpoint of growth regulation and organization in different plants, but it may be useful in applying to the general problem of transport of materials in plants. In the case of plant auxins and the synthetic compounds used in this laboratory, the bending responses constitute a much more sensitive test than has generally been

used for detecting the movement of mineral and elaborated substances in the plant. For example auxin A is effective in 1 part of auxin to 110,000,000 parts of water (3, p. 24) and ethylene is effective at a dilution of 1 part to 658,000,000,000 on a tissue weight basis (6, p. 239). Hetero-auxin (indoleacetic acid) is nearly as effective as auxin A in causing curvature of *Avena* coleoptiles, the value recently given by Kögl and Kostermans (11, p. 202) being 8 to 50 billion AE/g. which is equivalent to 1 part of hetero-auxin to 58,000,000 parts of water. When injected in a tomato petiole, less than 0.0001 mg. of indoleacetic acid will cause an epinastic response of the leaf (8, p. 92). Were similar methods available for detecting the presence and movement of small quantities of mineral and elaborated substances, it is believed that a clearer picture of the whole problem of transport of materials in plants might be obtained. Technique and methods of experimentation which require long periods of time introduce complications which are not encountered in tests with growth substances. Most of the data on the transport of normal plant constituents deal with the accumulation of materials over relatively long periods of time (several days or several weeks) and give little information on the channels, methods, or rate of movement.

With respect to soil treatments, the possibility seems likely that the synthetic growth substances moved to the aerial parts in the transpiration stream. Absorption of these substances was definitely dependent upon the rate of transpiration, but noticeable differences were obtained only when water loss from the plant was greatly reduced by placing the plants under bell jars in reduced light or in darkness. These results are important not only because they show that transpiration influenced absorption, but because they illustrate how efficient the absorption system is, even when the plant loses relatively little water. It is doubtful whether this minimum value for absorption has been reached in tests which appear to show that absorption of mineral nutrients from the soil is independent of transpiration. The source of supply of the substance in question was also an important factor in these tests. With minimum active amounts of the synthetic compounds, absorption of a sufficient amount to induce bending responses did not occur when the plants were placed in a high relative humidity or in darkness, or both. However, the use of larger amounts of these compounds showed that the bending responses occurred eventually on plants under bell jars in darkness. No doubt a relatively rapid disintegration of the growth substances occurs in soil. This is evidenced by the appreciable loss in activity which occurred during five hours in the case of plants left under bell jars for this period and then transferred to conditions of high evaporation.

The rate of movement of the synthetic growth substances was too rapid to be accounted for by simple diffusion. Under optimum conditions

the rate of upward transport from the soil was 47 cm. per hour with no allowance made for absorption or reaction time. Such a rapid movement probably occurs in the transpiration stream. The possibility of moving only in the phloem or in the living cells of the xylem is precluded by results which showed that living cells were not essential for the transport of these synthetic compounds. The aid of protoplasmic streaming is therefore not required. Downward transport of the synthetic compounds as judged by bending responses was relatively rapid but it was slower than the upward movement. The actual difference in these rates was not measured. When water solutions of the growth substance were admitted through the cut surface of a stem or leaf, the longitudinal movement in either direction was influenced by transpiration. These results indicate that both upward and downward transport of the growth substances were in the transpiration stream. With lanolin treatments, longitudinal movement of the synthetic compounds appeared to be mainly outside of the transpiration stream since in this case atmospheric conditions did not influence appreciably the transport of these compounds. Presumably the main longitudinal transport of growth substances from lanolin preparations takes place in living cells of the xylem or the phloem in tomato plants, although it was shown that a slow upward transmission occurred through a dead stem region. A similar possibility cannot be applied to the tobacco because there is no appreciable longitudinal movement of the growth substances when applied as lanolin preparations. Furthermore, water solutions of the growth substance showed practically no movement through the bark of tobacco stems. Fast Green dye moved in both directions through dead stem tissue as well as through intact tissue, so that the synthetic compounds are similar to the dye in this respect. However, Fast Green dye is different from the growth substances, since it would not pass through the intact membranes of tomato roots, whereas the growth substances did.

The transport of growth substances is dependent to a certain extent upon the method of applying the material to the plant. The systemic responses obtained on tobacco as a result of soil treatment were not induced by applying lanolin preparations to the aerial part of the plant (8, 9). These results indicate that the substances in lanolin are unable to penetrate to the conducting tissues that were reached by the material added to the soil or when introduced through a cut surface (18). In the latter case, when the solution was introduced in the slit portion of a tobacco stem, movement was principally upward and directly in line with the cut portion as evidenced by epinasty of leaves and later by rooting. These results furnish additional evidence that upward movement of the growth substances is in the vascular system and presumably in the transpiration stream since living cells are not essential for its transport. Since restricting

the point of entry of Fast Green dye limited its movement in the same manner as has been described for the growth substances (18), the tobacco plant offers good possibilities for use in the study of one-sided or an otherwise restricted type of transport of materials. In this respect tobacco is like many woody plants.

The inhibition of lateral bud development on tobacco stems by means of water solutions of the synthetic compounds indicates that these substances are similar to the inhibitor of bud growth normally present in the plant. Certain differences with respect to the movement of the two types of inhibitors under special experimental conditions would appear to be due to differences in concentration or amount. No doubt the synthetic compounds used in these tests were introduced in much higher concentrations and in larger amounts as compared to the quantity of the inhibitor of bud development normally present in the plant. It seems likely that the latter substance did not pass through a dead stem region because it was not present in a sufficient quantity to accomplish such a movement. Neither type of inhibitor showed any appreciable longitudinal movement through the bark tissue of tobacco stems, although both passed readily through a segment of the central woody cylinder from which the bark had been removed.

The reduction of transpiration by removal of all leaflets delayed or prevented bending responses much the same as when intact plants were placed in high humidity in reduced light or in darkness. Delay in bending or the failure to bend in this case was not due to the inability of the stem or midribs to respond, because bending was readily induced by applying lanolin preparations of the growth substance to the stem or to the midribs. Lack of bending responses on the plants shown in Figure 1 B indicates that a sufficient amount of the growth substance was not absorbed from the soil to cause the bending responses shown in Figure 1 A. Even when lanolin preparations of the growth substance were applied to the stem of the defoliated plants, systemic bending was much slower and less pronounced than on intact plants. It would appear, therefore, that movement of the growth substance from the region of application to distant points was retarded by removal of the leaflets. These results do not preclude the possibility that the leaflets exert other influences besides the control of transpiration in affecting the movement of the growth substances. Even proliferation and rooting responses at the region where the lanolin preparations were applied to stem or petioles were much less pronounced on defoliated plants than on intact plants. Also, the defoliated plants which showed bending responses exhibited little or no recovery of stem or leaves, whereas similarly treated intact plants showed considerable recovery. These results indicate that the effectiveness of preparations containing the synthetic growth substances in causing formative responses

is dependent to a considerable extent upon materials in the leaflets and upon functions over which the leaflets exert a controlling influence.

Since the transport of the growth substances is dependent upon the method of applying the material, the question of the relative sensitivity of different tissues must be regarded from a new angle. Most of the localized responses previously referred to (6, 8, 18) were explained on the basis that certain tissues, particularly those in the most active region of elongation, were more sensitive than others. This applies to the gases as well as to the crystalline growth substances. In the case of gases, for example, the principal region of bending is at the base of the petiole on all except the young leaves at the tip, and stem bending below the extreme tip portion seldom occurs. These differences are probably not due entirely to relative differences in sensitivity, because by means of lanolin preparations of the crystalline growth substances nearly any part of the leaf and nearly any portion of the stem which is flexible can be made to bend. Furthermore, the direction of the bend can be controlled by the lanolin method. An explanation on the basis of relative sensitivity of different parts of a plant can hold only when it has been demonstrated that the cells which initiate a given response have been subjected to the same concentration of the growth substance for the same period of time.

The formative responses induced by the synthetic growth substances are growth responses, so that any condition which influences noticeably the growth of the plant would also be expected to affect the action of the growth substances. Thus the absence of a formative response does not necessarily mean that an active amount of the growth substance has failed to reach that part of the plant. It may be merely a question of the plant being deprived of materials essential for growth in that region. Low temperatures which noticeably retard or inhibit growth will also delay or prevent the formative responses from occurring (18). With respect to the movement of the growth substance from the point of application to the region of response, adverse growth conditions may also delay or prevent penetration or absorption of the synthetic compounds. These changes occur normally on different parts of the same plant. For example, local and systemic responses are much less pronounced when lanolin preparations of the growth substance are applied to the lower part of the stem near the soil line as compared to treatment of the upper part of the stem of tomato plants. Thus the changes which are associated with the normal ageing processes in tomato stems will determine the rate of penetration of the growth substances from the epidermis to the longitudinal channels of transport. No doubt changes in tissue permeability also occur in plants of the same age that are placed under distinctly different growth conditions. Likewise, it would be expected that once having entered the xylem in one part of the plant, the growth substances would be prevented from moving

toward the epidermis where there is tissue which obstructs its passage. Although some synthetic growth substances are more effective than others and one tissue is more sensitive than another, marked differences in the response of plants of the same and of different species are probably determined in most cases by factors which affect the movement of the growth substance from the point of application to the region of response.

Bending of the regions treated with lanolin preparations of the growth substances appeared to be independent of the rate of transpiration, but the rate of bending was more rapid in reduced light or in darkness than at higher light intensities. Responses at distant points resulting from lanolin treatments likewise appeared to be independent of transpiration, although bending responses above the point of application occurred sooner and were usually more pronounced than those below the region treated. Both transverse and longitudinal movement of the synthetic growth substances resulting from treatment of the aerial part of the plant with lanolin preparations are therefore not influenced noticeably by transpiration as is the absorption of these same substances from the soil and their movement when introduced as water solutions in the transpiration stream. These results indicate that longitudinal movement of the synthetic compounds may also occur outside of the transpiration stream, but that once introduced into the stream, the movement is more rapid. Although living tissue is not required for the longitudinal transport of these substances, it does not follow that movement in this direction fails to occur in living tissue.

It now appears that failure to obtain systemic bending and rooting responses with lanolin preparations on tobacco were due not to differences in sensitivity of the tissue but to lack of penetration into the principal longitudinal channels of transport. Responses at distant points on the tobacco were first obtained by introduction of a solution of the growth substance into cut surfaces (18). In this case the main responses were on the side where the solution was introduced. Results reported in this paper show that typical systemic bending and rooting responses on the tobacco were obtained with soil treatments. Since the growth substances will pass through intact membranes as well as through broken parts of a root system, the absorption of these substances from the soil should not be regarded as essentially any different from the absorption of other types of materials already present in the soil. It is believed, therefore, that at least in some cases the failure to obtain bending or rooting responses with active preparations of growth substance may be due to the failure of the substance to reach the tissue in question or in a sufficient amount to induce the response. This does not imply that some cells are not more sensitive than others to a given concentration of growth substance. As a matter of fact this appeared to be the case when the same solution of growth substance caused hyponasty of the upper leaves of tobacco plants and epinasty

of leaves lower down, regardless of whether the solution was injected in the individual leaves or was applied to the soil. Lanolin preparations likewise induce positive or negative bending according to the concentration of growth substance and the age of the tissue treated (18). There appears to be no reason why the factors which determine whether a certain part of the plant makes a positive or negative response are different for the synthetic growth substances and for the plant auxins. No doubt many of the reponses to plant auxins may be explained on the basis of concentration of auxin, relative sensitivity of the tissue, and ease of penetration and distribution according to the method of application.

The noticeable influence of light on stem bending which makes it possible to detect a formative response in from 30 to 40 minutes after treatment, indicates that the mechanism involved may be similar to that present in *Avena* coleoptiles (4). This and a number of other similarities between the effects of auxin A and the synthetic growth substances is somewhat difficult to understand in view of certain pronounced differences. It seems possible that some of the differences may be more apparent than real. No doubt more work should be done with larger amounts and higher concentrations of the purified auxin A to determine whether or not it would act more nearly like the indole, naphthalene, and phenyl compounds when applied to *Avena* or to such plants as the tomato and tobacco. On the other hand, it is believed that some of the non-auxin compounds might be active on *Avena* were different methods of application used and a sufficiently high concentration employed. Even with the agar-block method it would seem that some of the indole (other than indoleacetic acid) and naphthalene compounds should induce bending of *Avena* coleoptiles if a high enough concentration were used. So much interest has been centered on the sensitivity of the *Avena* response that the possibility of high concentrations of auxin A inducing other types of physiological responses has been neglected. The use of high concentrations (1 to 10 per cent) of the compounds worked with in this laboratory has revealed a large number of interesting plant responses that would otherwise be unknown had only low concentrations been used. On the other hand, failure to work with low concentrations of growth-promoting substances has not been a policy of this laboratory since in the case of ethylene gas it has been shown that the African marigold is sensitive to 1 part of the gas to 60,000,000 parts of air (6, p. 240).

Further experimentation with hetero-auxin will no doubt be the means of learning more about growth responses in general than will result from tests which deal only with the true plant auxins, A and B. Hetero-auxin is of especial interest because its action on *Avena* is similar to auxin A and auxin B (15, p. 190), and its action on such plants as the tomato and tobacco is similar to a number of synthetic compounds, one of which

(indolepropionic acid) does not appear to be active on *Avena* (8). Hetero-auxin, therefore, forms a bridge between these two classes of substances, and from the standpoint of the bending response between two types of plants. Certainly hetero-auxin and a number of other synthetic growth substances are capable of performing some of the growth functions attributed to auxin A.

The results of Avery's tests with hetero-auxin on *Nicotiana* (1) represent a situation which is difficult to explain solely on the basis of the role which auxin A plays. The inferred additive effect induced in tobacco leaves by a lanolin preparation of hetero-auxin (1, p. 327) indicates that either of these auxins is regarded as being capable of causing the leaf to bend if applied locally at the base of the midrib where the natural supply of auxin was shown to be greatest. The hetero-auxin had less effect when applied to the lateral veins (1, p. 327), yet polar movement of auxin A from the lateral veins to the base of the midrib was postulated according to the diffusion test (1, p. 322). The assumption is that hetero-auxin showed the same polar movement as auxin A, although measurements of its movement were apparently not made. From our own results this difference in the degree of bending can be explained in two ways. In the first place hetero-auxin does not show a polar movement in *Nicotiana*, and any movement depends upon the method of applying the substance to the plant. Lanolin preparations of hetero-auxin generally cause only local bending responses on *Nicotiana* because the growth substance cannot penetrate readily to the channels of transport. However, a rapid transport of hetero-auxin occurs when a water preparation is added to the soil or is injected in the base of the petiole. In the latter case movement from the midrib to the lateral veins was evidenced by inward rolling of the margins on the young upper leaves and an arched bending of the midrib which constituted a hyponastic response. The physiological responses induced on *Avena* by the three auxins are admitted to be indistinguishable from one another (15). Since, however, hetero-auxin does not show a polar movement in the tomato and tobacco, the question arises as to whether the diffusion test for the transport of auxin in grass coleoptiles is really a measure of the movement that normally occurs in an intact sheath of *Avena* or in the leaves and stems of any other plant. Particularly is the diffusion test inapplicable to cases in which movement of growth substances is in the transpiration stream. Agreement concerning the movement of auxin in roots has not been reached. Du Buy and Nuernbergk (4, p. 349) are inclined to the view that no strict polarity of movement exists in roots, whereas Cholodny (5, p. 529) believes that basal transport occurs in roots as in the *Avena* coleoptile. According to the latter (5, p. 526) special precautions in the technique must be observed in order to demonstrate polar transport of auxin in roots. Improved methods for de-

termining the transport of auxin are probably needed to clear up this point.

The efficiency of the soil treatment method for inducing roots on tobacco stems suggests the possible practical application to woody plants, particularly those which do not root readily from cuttings. Although it may not be possible to induce an abundant growth of roots on intact potted woody plants, it seems probable that some roots might be initiated in young shoots if the transport of the growth substance to these regions could be effected. Removal of the young shoots from the parent plant after a suitable time and placing them in a rooting medium might then show that roots had been initiated as a result of the treatment. This method is now being tested on a few of the woody plants which have young shoots. Considering the present scarcity of these growth substances and the relatively large amount of the substance which is required for a single application, the soil treatment method is definitely limited in its use on potted plants, or plants grown in the field. Until more information is available, it would appear that the use of lanolin preparations of the growth substances is a more practical method for inducing roots on woody plants than the soil treatment method. The Japanese maple and the Grimes Golden apple (9, p. 363) and certain species of ornamental flowering apples have been rooted as a result of treatment with lanolin preparations of indoleacetic and indolepropionic acids. In this case the treated shoots were removed from the parent plant 7 to 21 days after treatment, and were then placed in the rooting medium.

The slightly earlier flowering of Turkish tobacco resulting from the soil treatments constitutes an additional physiological effect which the synthetic growth substances are capable of inducing. It now appears that these synthetic compounds are capable of exerting a controlling influence on the growth of stems, leaves, flower buds, vegetative buds, and roots—namely, on all major organs of the plant. The influence of these substances on organization processes has been established only for the initiation of roots and intumescence-like proliferations. Premature flowering of Turkish tobacco appeared to be due to the hastening of the terminal growth after the flower buds were formed. Although the latter assumption seems most probable in the tests referred to, the possibility seems likely that substances will be found which are more or less specific for the initiation of flower buds, at least in certain species of plants. Reference should here be made to the work being done with the animal sex hormone preparations which indicates that they induce earlier flowering on the hyacinth and certain other plants (12). Our results with the Turkish tobacco therefore confirm to a certain extent the results obtained with the sex hormones.

SUMMARY

1. The application of hetero-auxin, indolepropionic, indolebutyric, naphthaleneacetic, phenylacetic, and phenylpropionic acids to the soil

induced responses on the tomato and tobacco which were similar to the responses previously described as caused by the application of the same substances in lanolin, water, or oil to the aerial part of the plant.

2. Bending, proliferation, and rooting responses on stems and leaves and the inhibition of lateral bud growth were used as a means of detecting the presence of the growth substances in tissue some distance from the region of application. This method was used to determine the relative rates of absorption from the soil and movement through the aerial parts of the plant.

3. With minimum active amounts of the growth substance, absorption from the soil was delayed or prevented under conditions which greatly reduced water loss from the plant. With larger amounts, absorption was delayed but not prevented, even when plants were under bell jars in the dark. The amount of the growth substance used was, therefore, an important factor in demonstrating the influence of transpiration on absorption under extremes of atmospheric conditions. Absorption was effected through intact roots as well as through injured roots.

4. Responses induced by lanolin preparations of the same growth substances applied to aerial parts were not influenced by atmospheric conditions in the same manner as the responses resulting from soil treatment. In this case plants under bell jars or in reduced light or darkness usually responded sooner than those in the open in good light. Transverse and longitudinal movement of the growth substances from lanolin preparations on the epidermis were not noticeably influenced by transpiration as was the absorption from the soil described in item 3, or the movement referred to in item 8. Apparently longitudinal movement may also occur outside of the transpiration stream.

5. Since the type and degree of formative response depended upon the method of applying the growth substance, it was concluded that the true sensitivity of an organ or tissue to the growth substance cannot be determined until the factors affecting penetration and transport of the substance from the region of application to the region of the response have been accounted for properly. Thus, light, temperature, or relative humidity may become limiting factors for bending and rooting responses both before and after treatment of the test plants with the synthetic compounds.

6. Under optimum conditions the rate of upward movement of the synthetic growth substances in the tomato was in excess of 47 centimeters per hour. Living cells were not essential for the transport of these substances since movement in both directions occurred through dead tissue. Upward transport was faster than downward transport, and in either case the movement was more rapid through intact tissue than through dead tissue. There was no appreciable longitudinal movement of the synthetic compounds through the bark of tobacco stems.

7. A strictly polar movement of the growth substances was not observed in either stems or leaves of the tomato and tobacco.

8. It is believed that when the synthetic growth substances are applied as water solutions, they move longitudinally in the xylem, presumably in the transpiration stream since their movement was influenced by transpiration the same as their absorption from the soil. Success in producing responses at distant points with active water preparations of the growth substance seemed to depend upon whether penetration to the transpiration stream had been effected.

9. With respect to the lack of a strictly polar transport and the ease of movement throughout the plant, the crystalline growth substances are similar to ethylene gas.

10. Bending of stems constituted the most sensitive response to the growth substances. Under optimum conditions noticeable stem bending on tomato plants became evident in 30 to 50 minutes after treatment. Stems bent sooner (toward light) when one-sided illumination was furnished than when the source of light was not distinctly one-sided. Light, however, was not essential for stem bending since this response occurred also in darkness. The stem response to light was not reported previously.

11. Flowering of Turkish tobacco was hastened by applying growth substances to the soil three to five weeks before the controls flowered. A retarded growth was not associated with premature flowering. Optimum amounts for flowering were considerably less than optimum amounts for rooting. The influence of these particular growth substances on flowering has not been reported before, but other workers have shown that a similar effect is produced by animal sex hormones. The results with the synthetic compounds confirm to a certain extent the work with sex hormones on the hyacinth and certain other plants.

12. The bearing which these results have on the absorption and transport of materials in general and on certain practical applications with reference to the rooting of cuttings is discussed.

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FACTORS INFLUENCING GERMINATION AND DEVELOPMENT OF DORMANCY IN COCKLEBUR SEEDS

NORWOOD C. THORNTON

The dimorphic seeds of the cocklebur, *Xanthium canadense* Mill. (*X. pennsylvanicum* Wallr.) have for the past half of a century commanded the attention of many investigators. The non-dormant condition of the embyro of both the upper and lower seeds at the time of ripening suggests nothing unusual about the seed. However, upon examination one finds that the upper seed does not germinate under the usual growing conditions until many, months, and often years, after the lower seed.

Arthur (1) was the first to investigate the delay in germination of the upper seed which he attributed to a "hereditary character residing in the protoplasm of the embryo." Later Crocker (2) considered that this delay in germination, or dormancy, was due to the seed coat restricting the supply of oxygen to the embryo since both the upper and lower seeds germinated with equal rapidity when the coats were removed. He further showed that conditions of high oxygen, high temperature, constant or alternating, and coat injuries would bring about the germination of the upper seed as readily as the lower. Crocker attributed the delay in nature mainly to the seed coat effect, but did consider that the bur was effective in so far as it would aid in resisting seed coat disintegration. More recently McHargue (8) and Symons (13) published data to show that the bur was the factor in the retardation in growth of the upper seed in nature, but they apparently overlooked the possibility of minute injuries to the seed coat when removing the upper seed from the bur.

Shull and Davis (12) studying the catalase activity of the cocklebur seeds made an observation that provided the basis for an explanation of how the seed might remain viable in the soil, but without germinating, for a number of years. They found that the catalase activity of the moist seed was greatly reduced during storage. Ota (9), experimenting with seeds under moist conditions, found that the rate of respiration was greatly reduced with extended periods of storage. Davis (4) was able to induce dormancy in the cocklebur embryos by embedding the moist seeds in clay or burying in agar for two months at 27° to 30° C. under which conditions presumably a low oxygen supply was available to the seed. The dormancy of the embryo was overcome by subsequent moist storage at 5° C. for a period of three months. Davis found in nature that the embryos of the upper seed became dormant during the summer and after-ripened during the winter. This process could take place over a considerable period of time until the seed coat disintegrated sufficiently to admit sufficient

oxygen for germination. These observations explain how the cocklebur seed could have remained viable in Arthur's garden over a period of three or more years.

The dormancy *induced in the embryos* of the upper and lower cocklebur seeds by these gas treatment methods must not be confused with the delay in germination of freshly-harvested mature upper seed which are unable to germinate merely because the seed coat retards the passage of sufficient oxygen for germination; removal of the coat permits prompt germination showing that the embryo is in no way dormant. With the seeds treated by these gas treatment methods, however, germination does not occur at once even upon removal of the coat, showing that the embryo itself has gone into a dormant condition.

MATERIAL AND METHODS

The mature autumn harvested cockleburs were obtained from Kansas in 1930, 1932, 1933.[1] This species of seed is presumably the same as that used by Davis (4) and Shull and Davis (12). Each year within a few weeks after receiving them in Yonkers they were opened and the upper and lower seeds, as determined by their position in the bur, were removed and held separately. The seeds were then placed in distilled water at 5° C. for 12 to 16 hours. The fully imbibed seeds were examined for imperfect coats by the aid of a hand lens and those having defective coats, either cuts or natural imperfections, were not used except in special tests to be discussed.

The moist seeds were washed with running distilled water to remove fungous spores. By this method spores were easily removed from the smooth coat of the imbibed seed so that the experimental results were not affected by sterilizing agents.

In these experiments the seeds were placed on moist absorbent cotton in flasks which had been previously sterilized in an autoclave at 15 pounds' pressure for 20 minutes. When held for germination tests or moist storage in air the seeds were placed on moist absorbent cotton in petri dishes.

The gas treatment chambers or germinators consisted of Erlenmeyer, Kjeldahl, and distilling flasks of 1 liter capacity. The gases, CO_2, O_2, N_2, and H_2, used were obtained from commercial cylinders. In preliminary tests in order to obtain a desired gas mixture in the flasks containing the seeds, a large quantity, normally 9 to 16 liters, of the desired mixture (previously prepared by displacement with water) was forced slowly through the flask by means of capillary inlet and outlet tubes. This method was later replaced by a vacuum procedure described by Denny (5). The

[1] The writer wishes to express his appreciation to Professor W. E. Davis, Kansas State College of Agriculture and Applied Science, Manhattan, Kansas, for collecting the cocklebur seeds used in this study.

apparatus used to purify and deliver the gases was modified to eliminate the use of mercury by using water columns and a hollow spring type dial gauge. The flasks containing the seeds were evacuated (approximately 750 mm. pressure) four successive times; releasing the vacuum three times with nitrogen washed with alkaline pyrogallol, and the fourth time with the desired gas mixture. This procedure was carried out with different lots of seeds as frequently as once a day and as infrequently as once every 18 days. Irrespective of the presence or absence of the seed coat the treatment had no apparent detrimental effect on the embryo.

The majority of the experiments with the seeds were conducted in constant temperature ovens ranging in temperature from 21° to 35° C. However, special conditions were required for some tests and these will be discussed with the results.

EXPERIMENTAL RESULTS

OXYGEN MINIMUM FOR GERMINATION OF THE UPPER AND LOWER SEEDS

The oxygen percentages necessary to permit germination of cocklebur seeds are shown in Figure 1 (which gives the results with naked embryos), and in Figure 2 (which gives the results with seeds with coats on).

Naked embryos (see Fig. 1). With the experiments at 21° C. the lower seeds gave no germination with less than 0.6 per cent O_2, and the upper seeds required 1.1 per cent. With the experiments at 30° a similar relation between lower and upper seeds was found, but definitely lower percentages of oxygen were effective for both types of seed. That is, at 30° the minimum was reduced to 0.2 per cent O_2 for the lower and to 0.7 per cent for the upper.

Seeds with intact coats (see Fig. 2). Very much higher percentages of oxygen were required for the seeds with intact coats than with the naked embryos. Thus at 21° the minima for lower and upper seeds were 6 per cent and 60 per cent respectively and at 30° C. the minima were 4 per cent for the lowers and 30 per cent for the uppers.

Discussion of O_2 minimum. Figures 1 and 2 also show a distinct difference between upper and lower seeds in the range between the oxygen required to initiate germination and that needed to produce 100 per cent germination. The range was narrower for the lowers than for the uppers, and this is especially striking in the experiments with intact seeds (see Fig. 2). Thus during six days in the germinator at 21° C. no germination of the lowers was obtained at 1, 2, 3, 4, or 5 per cent O_2 while 6 per cent O_2 gave 100 per cent germination, and at this temperature the range for the uppers was 60 to 100 per cent O_2. At 30° the range was 4 to 6 per cent for the lowers and 30 to 80 per cent O_2 for the uppers.

The period of six days was considered best since maximum germination was obtained with the least interference with the maintenance of the

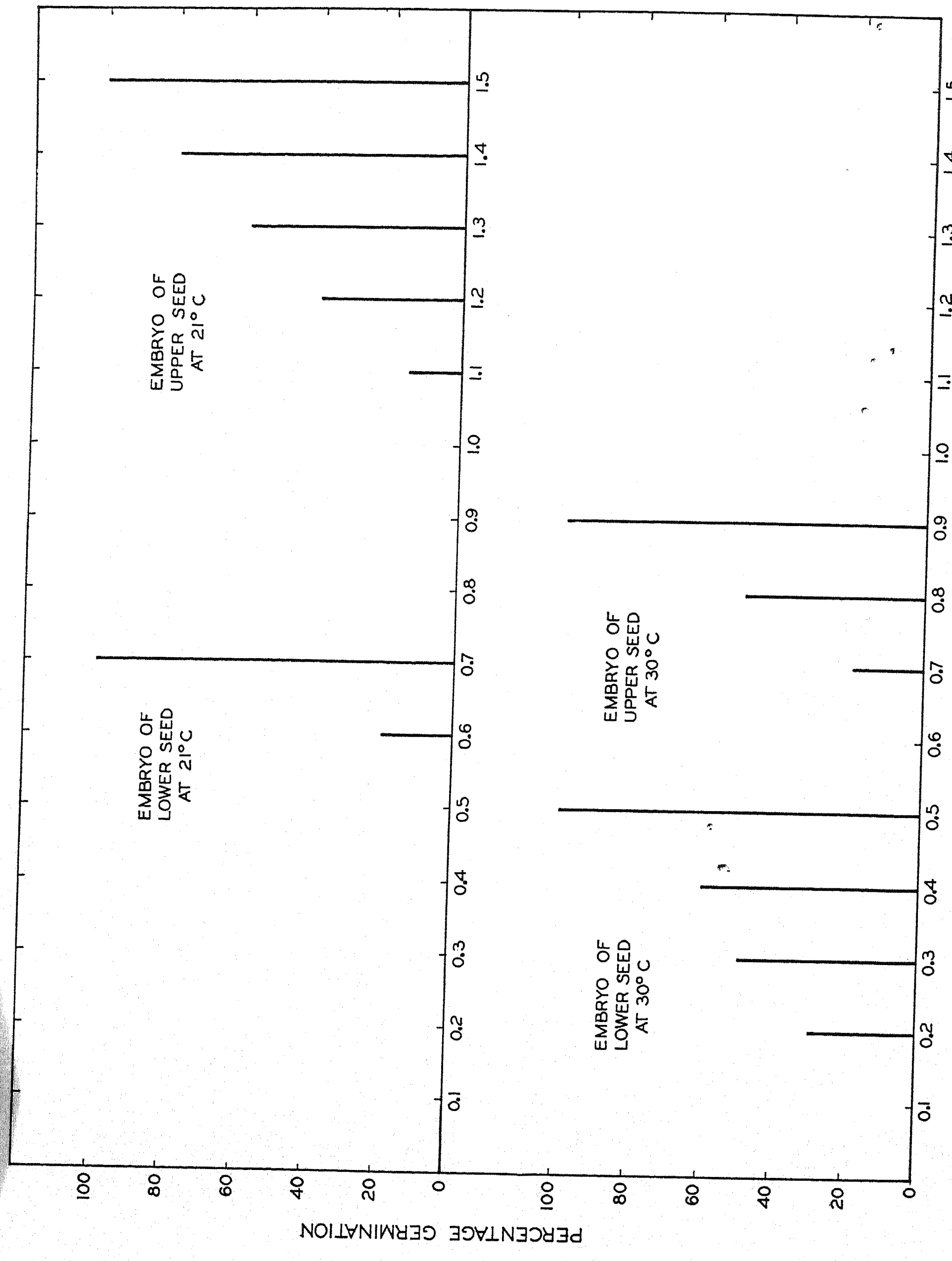

FIGURE 1. Minimum oxygen required for germination of the naked embryos during six days at 21° and 30° C.

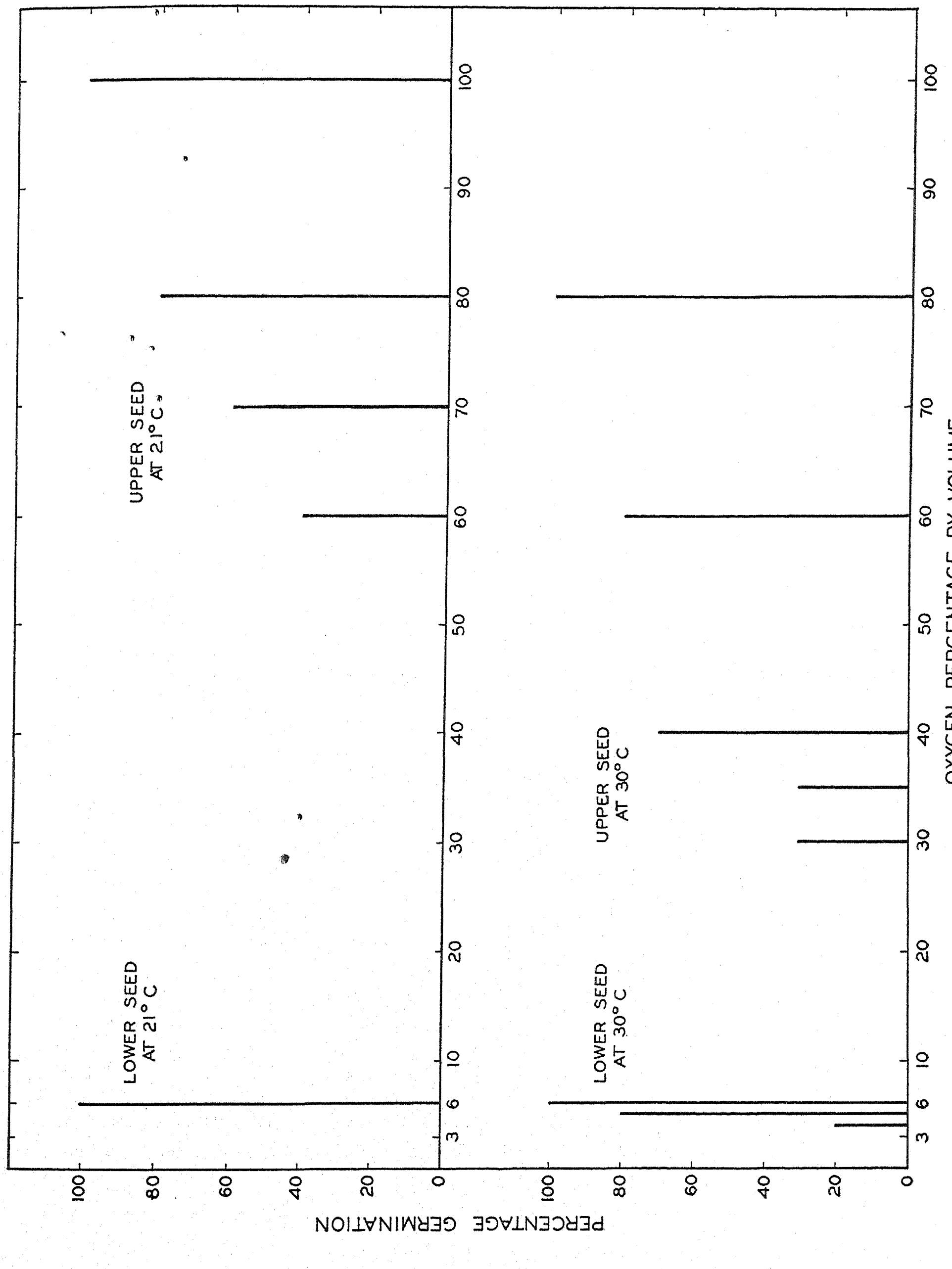

FIGURE 2. Minimum oxygen required for germination of the intact cocklebur seed during six days at 21° and 30° C.

oxygen supply by the growth of germinated embryos. Continued daily replacement of oxygen supply for additional periods in the germinators (a maximum of 14 days) did not greatly alter the percentages of germination as already given in Figures 1 and 2. Seeds which did not germinate even after the maximum period in the germinators did so and grew rapidly when the naked embryos were placed on moist cotton in petri dishes.

The upper seeds with coats intact germinated abnormally (elongation of the cotyledon before the radicle as shown in Fig. 3) in all effective percentages of O_2 at 21° C. and in 40 per cent and higher percentages of O_2 at 30° C. In 30 per cent O_2 at 30° C. half of the seeds that germinated did so in an abnormal manner while the other half that germinated did so in a normal manner (growth of the radicle before the elongation of the cotyledons). With an increase in the temperature to 35° C. all percentages (20 to 100) of oxygen brought about normal germination of the upper seeds with coats intact. Unlike the upper seeds the lower seeds, if they germinated at all, germinated normally in all percentages of oxygen at any temperature studied.

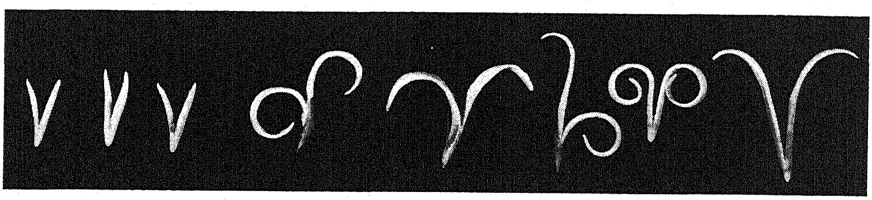

FIGURE 3. Development of the upper seeds of the cocklebur with coats intact in the presence of 80 per cent of oxygen during two days' germination at 28° C. Left to right: (seeds 1, 2, 3) control imbibed embryos freshly removed from the seed coats; (seeds 4, 5, 6) seeds exposed to O_2 showing the elongation of the cotyledons and the partially split seed coat closely covering the radicle; (seeds 7, 8) broken coats removed from the embryos showing the absence of growth of the radicle.

FORCING THE GERMINATION OF COCKLEBUR SEED

The intact moist upper and lower seeds of the cocklebur may be forced to germinate rapidly by exposure to high partial pressures of oxygen and to mixtures of carbon dioxide and oxygen.

Forcing with oxygen. At 25° C. O_2 pressure of 80 to 100 per cent of a full atmosphere forced complete germination of intact imbibed upper seeds within three days. With O_2 pressures ranging from 60 to 40 per cent of an atmosphere six to ten days were required for complete germination of these seeds. Increasing the temperature above 25° C. shortened the time required for germination of the upper seeds in any given O_2 pressure but the minimum O_2 pressure required for complete germination did not

fall markedly until the temperature was raised to 33° C. Only at this higher temperature did the intact upper seeds germinate in 20 per cent O_2.

The lower seeds with intact coats when exposed to 100 per cent O_2 pressure at 25° C. germinated completely within 16 to 20 hours. With decreasing percentages of O_2 the rate of germination of the seed was decreased until there were required approximately 72 hours for complete germination in 20 per cent of oxygen.

Forcing with carbon dioxide. At 25° C. CO_2 pressure of 80 per cent with O_2 pressure of 20 per cent of a full atmosphere forced the germination (80 per cent in four days and 100 per cent in six days) of the intact imbibed upper seeds. Lower pressures of CO_2 either 60 or 40 per cent with 20 per cent of O_2 forced 40 per cent germination of the intact upper seeds in four days and complete germination within seven days. With a reduction in the O_2 to 10 per cent, 40 per cent of CO_2 was the most effective concentration to force germination (40 per cent of the seeds germinated within four days and complete germination was obtained in eight days). Higher or lower pressures of CO_2 with 10 per cent O_2 pressure were not markedly effective in forcing germination of the upper intact seed. Increasing the temperature to 28° C. hastened germination but did not alter the relative effectiveness of the various gas mixtures.

TABLE I

EFFECT OF CARBON DIOXIDE ON THE RATE OF GERMINATION OF LOWER COCKLEBUR SEED WITH COATS INTACT AT 25° C.

Treatment, per cent of			Percentage germination, after hrs.			
O_2	CO_2	N_2	24	31	48	72
20	0	80	10	20	90	100
20	10	70	30	70	100	100
20	20	60	30	70	100	100
20	40	40	10	70	100	100
20	60	20	0	0	60	100
20	80	0	0	0	20	80*

* 100 per cent germination in 96 hours.

Treatment of the lower intact seeds with 10 to 40 per cent CO_2 with 20 per cent O_2 at 25° C. more than doubled their rate of germination as shown by the data in Table I. With higher percentages of CO_2 germination of the seeds was retarded for approximately 31 hours after which germination took place at a slow rate. However, complete germination was obtained in 80 per cent of CO_2 within 96 hours.

Germination of either the upper or lower seeds was never obtained in 100 per cent of carbon dioxide, nor was the rate of germination affected (as compared with control seeds) upon removal of the treated seed to normal air.

Discussion of forced germination. The germination of the upper and lower intact seeds forced with CO_2 proceeds in a normal manner. The growth of the radicle following the breaking of the seed coat is at first greatly retarded and finally inhibited on the third or fourth day of storage when injury becomes apparent. When the uninjured germinated embryos are removed to normal air further growth takes place at a rate comparable with the control seeds.

The effectiveness of oxygen in forcing normal germination of the lower and abnormal germination of the upper seeds has been mentioned previously and shown in Figure 3. The radicle of the upper seeds did not begin to grow until after the cotyledons had increased from two to three times their original size. As Crocker (2) found, oxygen is more readily available to the distal region of the cotyledons because of the thinness of the seed coat at this point. The portion of the coat covering the radicle is comparatively thick and offers some resistance to the passage of oxygen. A similar abnormal growth response of the less sensitive portion of the seed is readily obtained if the distal end of a moist upper seed coat is cut with a knife and germination is allowed to take place in a petri dish at room temperature. Under these conditions the growth of the upper embryo begins in an abnormal manner and a period of two to four days is necessary (after the coat is completely removed) for readjustment after which time normal growth takes place.

SEED COAT VERSUS MECHANICS OF THE BUR IN CAUSING THE DELAY IN GERMINATION OF THE UPPER SEED

Regardless of previous experimental work there still occur in the literature reports that the bur and not the seed coat is responsible for the delay in germination of the upper seeds in nature. Tests made on this phase of the problem show again that these investigators apparently injured the seed coat when removing the upper seed from the bur.

Imbibed upper and lower seeds carefully selected by the aid of a hand lens for freedom from scratches or imperfections were planted one inch deep in garden soil in the greenhouse at 27° C. Seedlings from lower seeds emerged from the soil 100 per cent within three weeks while the seedlings from the upper seeds did not begin to emerge until after nine weeks. During 51 weeks in the undisturbed soil the upper seeds with uninjured coats gave only 14.3 per cent emergence. Seeds having the waxy coat slightly roughened emerged more rapidly although the upper seeds displayed considerable delay even after this treatment. Germination of seeds in this condition was not uniform showing that some of the testas had been injured more than others. Seeds, either (a) chipped with a knife, (b) with coats pricked, (c) deformed, (d) with indentation in coat though the coat apparently was not broken, or (e) with coats having blackened particles

of dead tissue clinging to the deformed areas, were planted and in every case both the upper and the lower seeds emerged at nearly the same rate within two weeks. Seeds having defects mentioned under items c, d, and e above are responsible for the emergence of the upper seeds even when in the bur thus accounting for the occasional finding of the growth of two seedlings from one bur in nature.

Selected cocklebur seeds, uppers and lowers, were next planted in quartz sand in an oven at 28° C. The lower seed emerged within a period of ten days, while only 25 per cent of the upper seed emerged within 154 days. The ungerminated upper seed developed a dormant condition which is most likely the condition of the seed that McHargue (8) considered to be non-viable after being stored in moist sand at 28° C. for 90 days.

DEVELOPMENT OF DORMANCY IN THE EMBRYO OF THE COCKLEBUR SEED

Production of dormancy in the embryos of seeds by gas treatments. Holding intact imbibed cocklebur seeds for 46 to 86 days in atmospheres of nitrogen, hydrogen, carbon dioxide, or in various percentage mixtures of CO_2 and N_2 induced dormancy in the embryos. This is shown by the fact that seeds treated with these gases or gas mixtures germinated much more slowly than untreated seeds. Thus as shown in Table II the treated seeds needed 11 to 37 days for 100 per cent germination while the control lots reached this stage in 1 to 3 days.

The embryos of the seeds held at 28° C. during gas treatments were more dormant than those held at 21° C. When naked embryos from both the 28° and 21° lots were placed subsequently under the same conditions for germination at 21° C., the 21° lot germinated much sooner than the 28° lot (100 per cent in 37 days for the 21° lot and only 20 per cent in 60 days for the 28° lot). Tests of the germination of the naked embryos at higher temperatures, up to 35° C., likewise showed slower germinations for the 28° seeds.

Dormant embryos from gas treatments at 28° that required ten or more days for germination exhibited many of the growth characteristics of naked embryos of seeds such as *Ambrosia trifida* (3) which are naturally dormant at the time of harvest. This growth is considered to be abnormal since the cotyledons (in contact with the moist medium) enlarged, in some cases becoming green, and the plumule grew 2 to 4 mm. before the radicle started to grow as is shown in Figure 4 D. At the time the radicle started to grow the cotyledons ceased growth and the plumule suspended further growth until after the radicle was well developed. The radicle elongation, when started, continued for some distance as a rounded, thickened, blunt-like root as shown in Figure 4 D. After a few days, usually from four to six, varying with every seed, a pointed slow-growing radicle having a tendency to curl in an indefinite direction on the moist cotton developed into a normal-growing root.

The abnormal growth was produced by seed from the gas treatments at 21° C., but never to the same extent as was produced by seed from the

TABLE II

INDUCING DORMANCY IN THE COCKLEBUR SEED BY KEEPING IN GERMINATORS IN VARIOUS GAS MIXTURES

Seed	Treatment, per cent of			Temp. held during treatment and germination, °C.	Days in gas treatment	Days in petri dish after gas treatment	After gas treatment coats removed. Days for germination of		
	N_2	CO_2	H_2				25%	50%	100%
Lower	100			28	86	5	4	10	14
		100		28	86	5	3	7	12
			100	28	66	5	1	10	17
	90	10		28	86	5	5	8	14
	88	12		30	46	30	3	16	23
	80	20		28	66	5	1	2	11
	50	50		28	66	5	1	3	14
	20	80		30	46	30	16	21	30
	20	80		28	66	5	8	11	14
	Control*			28					1
Upper	100			28	66	5	2	4	11
		100		28	66	5	1	4	11
			100	28	66	5	2	7	16
	90	10		28	67	5	2	6	11
	85	15		28	66	5	9	11	20
	80	20		28	86	5	3	5	14
	70	30		28	67	5	1	4	14
	50	50		28	67	5	3	8	11
	20	80		28	67	5	2	3	11
	Control*			28				1	2
Lower	100			21	66	5	4	7	37
		100		21	66	5	3	5	25
			100	21	37	5	1	3	16
	90	10		21	66	5	2	6	11
	50	50		21	66	5	10	19	37
	20	80		21	66	5	7	15	35
	Control*			21				1	2
Upper	100			21	67	5	1	3	15
		100		21	37	5	2	7	17
			100	21	67	5	1	4	24
	90	10		21	67	5		1	11
	80	20		21	67	5	7	11	12
	70	30		21	67	5	2	11	17
	50	50		21	67	5	8	11	16
	20	80		21	67	5		1	18
	Control*			21				1	3

* Control seeds were removed from burs and soaked in water for 16 hours at 5° C. before the coats were removed for the germination test.

gas treatments at 28° C. In cases where cotyledonary enlargement took place it was not followed by growth of the plumule before the development of the radicle.

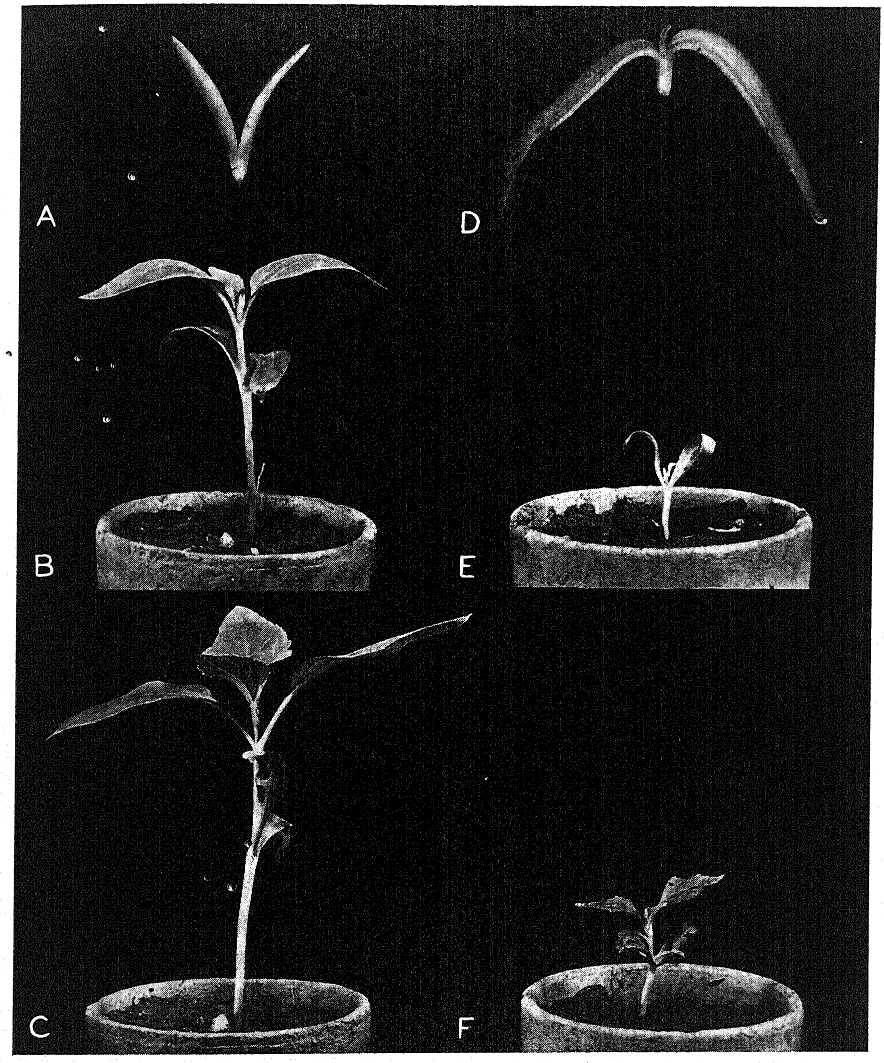

FIGURE 4. The enlargement of cotyledons and growth of plumule of seed with dormant embryo and subsequent production of dwarfed seedling from these seeds. (A) Untreated imbibed embryo of lower cocklebur seed. (B) Control seed after 14 days' growth. (C) Control seed after 21 days' growth. (D) Growth of treated embryo of lower seed held without coat under germinating conditions for 18 days (following treatment, with coats intact, of 52 days in pure H_2 and 209 days on moist cotton in air at 28° C.). (E) Dwarfed growth of seedling 14 days after planting. (F) Growth of seedling made in 29 days.

After-treatment conditions which induce further dormancy. In the experiments just described, only five days elapsed after the end of the gas treatment period before the naked embryos were placed under germinating conditions. When this period after gas treatment (during which the seeds were placed on moist cotton in petri dishes) was increased to 59 days before the coats were removed it was found that the dormancy of the embryos had increased. Thus, in Table III, gas-treated seeds whose coats were removed on the sixth day after the gas treatment, required about five to eight days longer for germination than untreated seeds, but when the period of treatment before the removal of the coats was extended to 59 days the delay in germination was increased to about 11 to 19 days.

TABLE III

INCREASED DELAY IN GERMINATION BROUGHT ABOUT BY MOIST STORAGE AFTER GAS TREATMENT*

Seed	Treatment, per cent of			Coats removed after 6 days in petri dish. Days required for germination of			Coats removed after 59 days in petri dish. Days required for germination of		
	CO_2	O_2	N_2	25%	50%	100%	25%	50%	100%
Lower			100	2	3	8	12	16	18
	50		50	1	3	7	6	12	15
	50	1	49		1	6	1	6	7
	100			1	3	6	8	9	12
	Control**					1			1
Upper			100		1	9	8	13	18
	50		50		2	6	8	12	20
	50	1	49		1	6	1	3	10
	100				1	7	6	7	10
	Control**					1			1

* Seeds held for 62 days at 31° C. in the various gases.
** Control seeds were removed from burs and soaked in water for 16 hours at 5° C. before the coats were removed for the germination test at 31° C.

In the first germination test (six days after removal of seeds from the gas treatment) in Table III, embryo dormancy was indicated only by the retarded growth of the radicle which in the usual manner preceded the growth of the cotyledons and plumule. However, in the second germination test (after 59 days' moist storage in petri dishes) the dormant seeds displayed considerable cotyledonary enlargement and plumule growth preceding the growth of the radicle. This latter type of growth as already discussed is characteristic of the growth of very dormant embryos.

As shown in Table III upper and lower seeds held in gas mixtures, containing 1 per cent of oxygen, developed a dormant condition comparable with those seeds held in oxygen-free mixtures. With the additional period of holding on moist cotton in air the upper seeds whose coats re-

strict the movement of oxygen to the embryo become slightly more dormant. The dormancy of the lower seed was not influenced by the additional period on moist cotton in air since the seed coat did not restrict sufficiently the movement of oxygen to the embryo.

Further consideration in inducing dormancy. Besides the gas mixtures given in Tables II and III many other combinations were used with equal effectiveness provided oxygen was absent. However, tests were made using besides 1 per cent, 7, 14, and 20 per cent of oxygen with CO_2, N_2, and H_2, and mixtures of these gases. With these mixtures containing high percentages of O_2 the flowing gas method was used with some success in the production of a slightly dormant embryo with as high as 7 per cent of O_2 where the gases were changed every two weeks. However, this procedure was unsatisfactory because of a loss of 40 to 60 per cent of the lower and 20 per cent of the upper seed through germination during the test. The vacuum procedure with zero or even 1 per cent of O_2 reduced this loss to a maximum of 10 per cent with an average of approximately 5 per cent for either the upper or lower seeds. In most cases this loss is due to breaking the coats (rarely did germination occur even with the lower seeds) shortly after the first vacuum replacement of the gas atmosphere at the beginning of the experiment. Usually from 2 to 5 per cent of the seeds, especially the lowers but also the uppers, did not develop a dormant condition even with prolonged treatment with the various gas mixtures. When removed to moist cotton in petri dishes both the lower and upper non-dormant seeds germinated within four days regardless of the presence of the seed coat. It is possible that some of the seed coats were injured during the transfer from flasks to the petri dishes. Because of this condition the seeds were always held with coats intact five or six days (Tables II and III) in the petri dishes after removal from the gas atmosphere before germination tests were made with naked embryos to show dormancy.

Catalase activity of dormant seeds. The catalase activity of the embryos held nine weeks in CO_2 or N_2 or in mixtures of these gases (without oxygen) was reduced to approximately one-half of that of the moist control. In tests where 1 per cent O_2 had been used the catalase activity was reduced to 20 per cent of that of the control. When held under conditions favoring germination the naked seeds regained approximately the original catalase activity preceding growth of the radicles.

Induced dormancy in embryos of seeds held in soil and quartz sand. Ungerminated upper seeds that had been in soil in the greenhouse for 51 weeks, as previously discussed, displayed considerable dormancy when the naked seeds were held on moist cotton at 30° C. Fourteen days were required for complete germination of these seeds. In 40 per cent of the germinating seeds the cotyledons enlarged and the plumule grew 2 mm.

before the radicle began to elongate. The size and shape of the radicle of most seeds were abnormal and the rate of its elongation was retarded, taking approximately five days to show normal growth. Apparently these seeds had become dormant through the reduction in oxygen supply at the minimum night temperature of 27° C. During the summer months these seeds were exposed to very much higher temperatures especially during the day.

Upper seeds held in moist quartz sand in a constant temperature oven at 28° C. for 22 weeks developed considerable dormancy in the embryos. When the coats were removed, nine days were required for complete germination of these embryos. It is evident that some dormancy can be produced in the embryo of the upper seed of the cocklebur without special requirements as to the oxygen supply because the upper seed coat retards the entrance of oxygen to the embryo.

Breaking the dormancy of the embryos of seeds made dormant by treatment. After-ripening of the embryos of these seeds with intact coats was accomplished by three months' storage at 5° C. Upon removal from storage the naked seeds germinated as rapidly as corresponding upper and lower control embryos at any temperature from 21° to 30° C. At 35° C. the after-ripened seeds with coats intact germinated as readily as intact imbibed seeds freshly removed from the burs. Unsuccessful attempts were made to force the germination of intact seeds with dormant embryos by the use of high percentages of O_2, by mixtures of CO_2 and O_2, and by the use of a temperature of 35° C.

Growth of plants from embryos previously made dormant by treatment. The cocklebur seedlings shown in Figures 4 and 5 represent the growth (in soil in greenhouse at a minimum temperature of 20° C. from February to April 1934) of the plants obtained by germination of the dormant embryos. Completely dormant embryos produce upon germination and growth, dwarf plants (Fig. 4 E, F) which in most cases had unnatural curly leaves as shown in Figure 5 A to D. The seedlings displayed this very slow rate of growth for varying periods of time. Seedlings from the least dormant embryos grew slowly for two weeks while the majority (having produced during germination cotyledon and plumule enlargement before the radicle) continued to grow slowly for as much as five weeks. At the end of the period of slow growth the plants, for the first time, produced leaves that developed to normal size and shape after which further growth took place at the same rate as the control plants.

The cocklebur plant shown in Figure 5 E corroborates the previous statements that the embryos of the seeds held at 21° C. during gas treatment are less dormant than those of seeds held at a higher temperature. This representative plant after 14 days' growth is nearly as tall as the control plant of the same age shown in Figure 4 B. The representative plant in Figure 5 F is indicative of the effect of the presence of oxygen

upon the intensity of dormancy produced in the seed since the plant is one-half as tall as the control plant of the same age in Figure 4 C.

Discussion of induced dormancy. It is evident that the conditions that Davis (4) obtained were essentially those of a minimum oxygen exchange

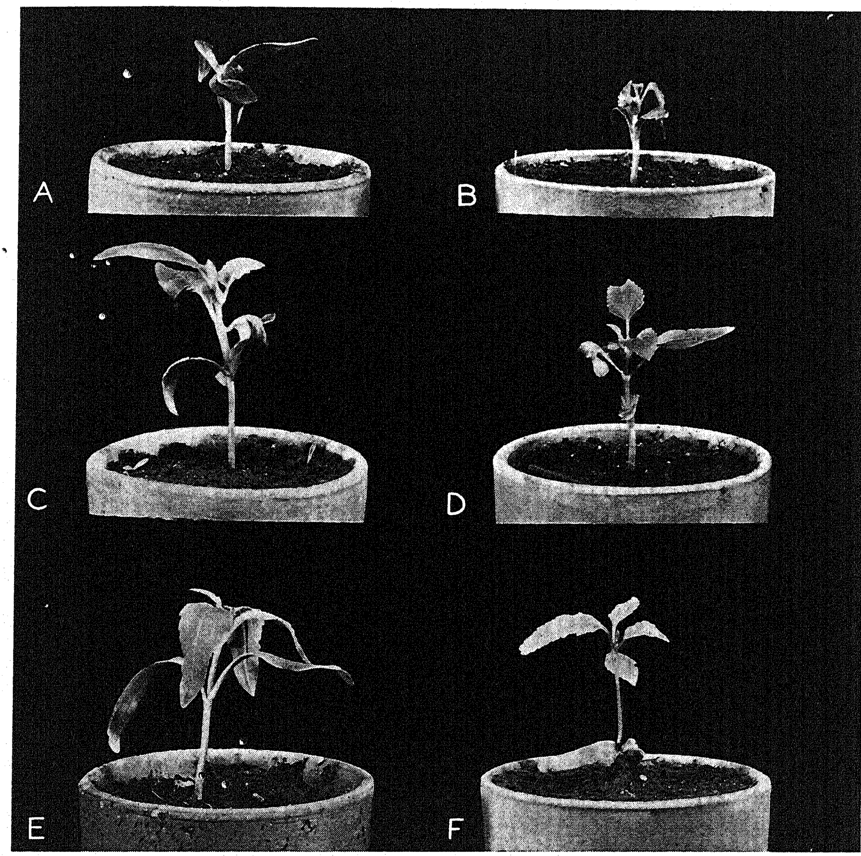

FIGURE 5. Seedlings from dormant embryos of upper seeds showing dwarfing and modified or curly leaves. (A, B) Dwarfed plants with modified leaves after 20 days' growth (seed treated with N_2) as compared with growth of control (Fig. 4 C). (C, D) Same as (A) and (B) after 29 days' growth. (E) Fourteen days' growth by plant from less dormant seed treated at 21° C. (F) Twenty days' growth of plant from seed treated with 50 per cent CO_2, 1 per cent O_2, and 49 per cent N_2 at 31° C.

with a possible accumulation of carbon dioxide within the embryo during the high temperature storage in clay or agar. In these experiments atmospheres of zero oxygen and 10 per cent carbon dioxide have given very good results in the production of the dormant condition. However, such a gas mixture is not considered to be superior to other oxygen-free mixtures or pure gases used in these tests. When selecting pure gases for long periods

of treatment the inert ones which have like effects such as hydrogen or nitrogen should be used in preference to carbon dioxide since the latter gas is not inert. It was found that at the end of four months' treatment the cocklebur seeds in pure carbon dioxide were of poor color and many were showing some physiological breakdown. Seeds stored for one to two months in this gas did not show any detrimental effects upon germination other than the previously discussed dormant condition. Carbon dioxide diluted with any amount of nitrogen did not bring about a condition of physiological breakdown even with the longest period of treatment.

DISCUSSION

In general, naked cocklebur seeds will germinate approximately the same under a given oxygen pressure whether this is by a partial vacuum with air or by a proper mixture of gases at full atmospheric pressure. Shull (10, 11) exposed naked embryos of upper and lower seeds to partial vacuum with air during germination tests of ten days at 21° and 31° C. and from the results he approximated by a mathematical method the oxygen minima for germination. At 21° C. the upper embryos required 12 mm. and the lower 9.5 mm. of O_2 pressure and at 31° C. the upper embryos required 7 mm. and the lower 3 mm. Transferring the data of Shull from the pressure basis to the basis of per cent by volume, we find that at 21° C. the upper embryo required 1.58 per cent of O_2 and the lower required 1.25 per cent, while at 31° C. the upper embryo required 0.92 per cent and the lower 0.39 per cent of oxygen for germination. These calculated data compare favorably with a direct measurement of the minima when using the proper mixture of gases at full atmospheric pressure. As shown in Figure 1, the upper embryos at 21° C. required 1.1 per cent of O_2 and the lower 0.6 per cent, while at 30° C. the upper embryos required 0.7 per cent and the lower 0.2 per cent of oxygen for germination. Although there is some difference in the actual data obtained, the results by either method show that the lower embryo has a lower minimum oxygen pressure for germination than the upper at either temperature. However, the most striking difference is in the lowered requirement of oxygen pressure for germination by the embryos as the temperature is increased from 21° to 30° C. This is a condition decidedly different from that usually found with seeds when the demand for oxygen increases with temperature.

The oxygen minima for germination of the cocklebur seeds are greatly increased by the presence of the seed coat. Comparing the results in Figures 1 and 2 we find that the O_2 minima at 21° C. have been increased, due to the presence of the coat, 10 times for the lower and 54.5 times for the upper seed and at 31° C. 20 times for the lower and 42.8 times for the upper seed. These results indicate that the seed coat is an important factor in the retardation of germination of the upper seeds in nature.

The failure of the upper seed to germinate in normal atmospheres con-

taining 21 per cent of oxygen at 25° C. leads one to consider closely the mechanism by which the same seed can be forced into germination by increasing the O_2 supply as suggested by Crocker (2) or with mixtures of CO_2 and O_2 such as used by Harrington (7) to force growth of dormant seed of Johnson grass. In the case of increased oxygen supply the effect is merely that of an increased partial pressure of oxygen in the atmosphere which overcomes the inhibiting effect of the seed coat by providing a steeper diffusion gradient between the outside and inside of the testa. With the carbon dioxide treatment, however, a greatly different process must be considered. In the presence of this gas the upper seed will germinate in 20 per cent or even 10 per cent of oxygen, and the germination of the lower seed may be greatly hastened by this same treatment. There is no doubt that the carbon dioxide must bring about a change in either the seed coat or the embryo, or possibly both. If the change is brought about in the seed coat it may be one of altered permeability which allows for a passage of oxygen through the coat to the radicle. In this case the penetration of oxygen must take place as readily at the point covering the radicle where the testa is thickest as at any other point covering the cotyledons since no abnormal germination has been observed here as with the cases of increased oxygen atmosphere, where the oxygen penetrates most easily at the distal end of the cotyledons where the testa is the thinnest. This theory of change in permeability of the testa has been strengthened by more recent observations on other plant tissue where the permeability is greatly altered by treatment with high concentrations of carbon dioxide. It is possible that CO_2 causes this change in permeability of the testa by inducing a chemical change in the tannin of the seed coat. An alternative suggestion is that the presence of the carbon dioxide brings about changes in metabolism of the embryo whereby less oxygen is needed to complete the chemical processes necessary for the growth of the radicle. In this case the gas may be considered as indirectly activating chemical changes within the living tissue so as to break down stored food materials, whereby the radicle may obtain sufficient growth impetus to break the testa, after which growth would proceed in a normal manner. This suggestion has been strengthened by the fact that a measurable decrease in hydrogen ion concentration may be obtained in the seeds preceding germination when they are held in the presence of carbon dioxide with oxygen.

The dormancy developed in the cocklebur seeds as a result of the gas treatments is within the embryo and is not a delay in germination due to the seed coat since the dormancy is evident even after the removal of the seed coat. This dormancy induced in the embryo must not be confused with the dormant condition exhibited by the untreated intact upper seed held at temperatures lower than 33° C. where the seed coat retards the passage of sufficient oxygen for germination to a non-dormant embryo. Whether or not changes take place in the seed coat during the develop-

ment of dormancy is not known. However, the seed coat does take part in the production of embryo dormancy through its limitation of gaseous exchange during and after the period of gas storage. This is as true with the lower as with the upper seeds under laboratory conditions for the production of dormancy but in nature it is only the upper seed coat that is effective enough to aid in the production of a dormant embryo. As Davis (4) has reported and as has been shown in this paper, dormancy can be expected to be produced in nature in the upper intact cocklebur embryo that does not germinate in the spring. The moist seed exposed to a high summer temperature with restricted oxygen supply due to its depth in the soil develops a dormant embryo. During the winter the embryo after-ripens and is again ready to germinate, provided that either the seed coat has disintegrated sufficiently to admit oxygen to the embryo, or that a sufficiently high temperature is available in the spring which would overcome the coat effect in retarding germination of the upper seed. If the seed does not germinate, another summer of high temperature will induce dormancy which is again broken by the low temperatures of winter. No experimental information is available as to how long such a process would continue, but Arthur (1) has reported that the spread in germination of seeds planted in garden soil (exposed to seasonal temperatures) extended over a period of three or more years. It is a question how many of our wild plant seeds follow this procedure in nature, thus spreading the germination of a crop over a period of many years with greatest germination in the spring of the year.

As previously reported (14) the intact upper and lower seeds with embryos made dormant by gas treatment (in the absence of oxygen) for one to four months at 28° or 31° C. have been held without germination for additional periods up to 20 months in petri dishes on moist cotton at the same temperatures. When the coats were removed from these seeds, however, germination of the embryos took place over a period of 25 to 35 days.

As Davis (4) reported the abnormal growth of the dormant embryo of cocklebur seeds (cotyledonary enlargement before growth of the radicle) when placed under favorable conditions is typical of the growth of some embryos naturally dormant at the time of harvest such as Davis (3) observed for *Ambrosia trifida*. The radicle appears to be much more affected than the cotyledons or plumules by the agents which bring about the dormant condition.

The growth of the seedlings produced by the dormant embryos is characteristic of the growth of normally dormant seeds. The seedlings (Figs. 4 and 5) show dwarfing, curling of leaves, and slow growth followed by a period of normal growth as reported by Flemion (6) for the germination and growth of the mature, yet dormant, embryos of seed of various species.

SUMMARY

1. The upper and lower seeds of cocklebur (intact seeds and naked embryos) require a lower partial pressure of oxygen for germination as the temperature is increased from 21° to 30° C. (see Figs. 1 and 2).

2. The oxygen, in per cent by volume, for germination of the cocklebur embryos and intact seeds within six days is as follows: naked embryos at 21° C., upper 1.1 per cent and lower 0.6 per cent; at 30° C., upper 0.7 per cent and lower 0.2 per cent; intact seeds at 21° C., upper 60 per cent and lower 6 per cent; at 30° C., upper 30 per cent and lower 4 per cent.

3. Germination of the intact imbibed upper seed of the cocklebur takes place in 20 per cent of oxygen only at a temperature of 33° C. or higher. Germination of these seeds at 25° C. can be forced by the use of 80 to 100 per cent of oxygen.

4. Germination of the intact seeds in oxygen takes place with the growth of cotyledons before the growth of the radicle rather than by growth of the radicle followed by an enlargement of the cotyledons as is the normal procedure.

5. The imbibed intact upper seeds germinate normally at 25° C. with as little as 10 per cent of O_2 only when a high concentration of carbon dioxide is present in the atmosphere.

6. The germination of imbibed intact lower seeds is hastened by holding in 10 to 40 per cent of carbon dioxide with 20 per cent of oxygen.

7. With 20 per cent of oxygen, no concentration of carbon dioxide was found to inhibit germination of intact seeds for more than 31 hours.

8. The bur plays no fundamental part in causing the delay in germination of the upper seed in nature. Those workers attributing the effect to the bur may have injured the seed coat upon removing the seed from the bur.

9. Dormancy is induced in the embryos of the upper and lower cocklebur seeds by placing intact seeds in flasks with atmospheres lacking oxygen but composed of N_2, H_2, CO_2, or various mixtures of CO_2 and N_2 for periods of 4 to 16 weeks at 28° to 31° C. The length of the dormant period induced in this way was increased by subsequent storing of the intact treated seeds on moist cotton in petri dishes for a similar period at 28° to 31° C.

10. Atmospheres such as N_2, H_2, CO_2, or mixtures of CO_2 and N_2 are rendered much less effective in inducing dormancy if as little as 1 per cent O_2 is present.

11. When the seeds were held in the various gas mixtures without oxygen at 21° C. instead of at 28° to 31° C. there was produced only a partially dormant condition which was easily broken and which did not increase with subsequent holding of the seed on moist cotton.

12. Partial dormancy may be induced in the embryo of intact upper seeds by storage in soil or sand at a temperature at least as high as 27° C.

13. During the treatment used to induce dormancy in the embryo the catalase activity is lowered.

14. Three months' moist storage of the intact seeds at 5° C. overcame the dormancy of embryos made dormant by the gas treatment.

15. The gas treatments which induce dormancy exert their effect upon the embryo itself since such seeds are unable to grow even when the seed coat is removed.

16. The subsequent growth of some embryos in which dormancy was induced by gas treatments was comparable to the growth of seeds (such as *Ambrosia trifida*) that have dormant embryos at the time of harvest. That is, growth of cotyledons and plumule starts before that of the radicle.

17. The seedlings produced by the germination of the dormant embryos show dwarfing, curling of leaves, and slow growth for a period of two to five weeks (Figs. 4 and 5) which was followed by a period of normal growth.

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CHEMICAL AND TOXICOLOGICAL STUDIES ON ORGANIC THIOCYANATES[1]

ALBERT HARTZELL AND FRANK WILCOXON

In previous publications (4, 8) from this laboratory it has been shown that the SCN group in organic combination confers high toxicity toward insects on a large number of compounds. The present paper is a continuation of these studies, in which certain thiocyanates have been compared with each other, and with other known or possible insecticides. In addition to this the action of thiocyanates as stomach poisons and the toxicity of thiocyanate spray residues to guinea pigs have been investigated.

COMPARATIVE TOXICITY OF TRIMETHYLENE THIOCYANATE AND LAURYL THIOCYANATE

These compounds were first compared at a concentration of 0.1 per cent, and emulsified with 0.5 per cent Penetrol. The method of testing was the same as described previously (3), using *Aphis rumicis* L. Subsequently, the compounds were tested at equal concentrations of SCN (0.03 per cent and 0.086 per cent respectively). The results of these tests are shown in Table I. From these experiments it appears that the two compounds are

TABLE I

COMPARATIVE TOXICITY OF TRIMETHYLENE THIOCYANATE AND LAURYL THIOCYANATE TO APHIS RUMICIS

Compound	Concentration of thiocyanate, per cent	Per cent dead
Trimethylene thiocyanate	0.1	90.0 94.9
Lauryl thiocyanate	0.1	90.4 95.6
Trimethylene thiocyanate	0.1	90.5 95.4
Lauryl thiocyanate	0.1	89.3 93.1
Trimethylene thiocyanate	0.03 (0.02% SCN)	54.7
Lauryl thiocyanate	0.086 (0.02% SCN)	83.0

not significantly different when compared on an equal weight basis. The molecule of trimethylene thiocyanate contains two SCN groups, while lauryl thiocyanate has only one. When the compounds were compared at equal concentrations of SCN, lauryl thiocyanate was the more toxic.

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 106.

A group of compounds closely related to the thiocyanates are the thiazoles, which contain the SCN group as part of a ring structure. Such compounds have been stated by Wetherill and Hann (7) to exhibit insecticidal action. For this reason 2-methyl-4-*p*-chlorophenyl thiazole was prepared as described by the above authors and tested in the form of the hydrobromide. Another sulphur-containing compound, thiodiphenylamine, was included in the tests. This compound has been found effective against mosquito larvae by Campbell and others (2). Butyl resorcinol which had been used previously in fungicidal experiments (9) was tested for contact insecticidal action. The first two compounds were ineffective when tested on *Aphis rumicis*, while the third compound caused severe injury to the nasturtium (*Tropaeolum minus* L.) foliage.

COMPARATIVE TOXICITY OF ROTENONE AND LAURYL THIOCYANATE

Since lauryl thiocyanate appeared to be the most satisfactory of the thiocyanates tested, it was compared with rotenone in order to find the relative toxicity of these two substances in pure form. As usual Penetrol was used as the emulsifying agent. Mixtures of lauryl thiocyanate and rotenone were also tested to see how the toxicity of the mixture compared with that of the components. The results are shown in Table II. It may

TABLE II

COMPARATIVE TOXICITY OF LAURYL THIOCYANATE AND ROTENONE TO APHIS RUMICIS

Compound	Concentration, per cent	Per cent dead
Lauryl thiocyanate	0.02	55.1
		53.1
Rotenone	0.02	70.0
		95.0
0.01% lauryl thiocyanate+0.01% rotenone	0.02	94.6
		93.9
Lauryl thiocyanate	0.0032	36.6
Rotenone	0.0032	82.2
0.0016% lauryl thiocyanate+0.0016% rotenone	0.0032	82.9

be seen that the thiocyanate is distinctly less toxic than rotenone. In the case of the mixtures of equal parts of thiocyanate and rotenone, the toxicity of the mixture was equal to that of a pure rotenone suspension containing an amount of rotenone equal to the total concentration of the mixture. The toxicity of the mixture was greater than that of a spray containing lauryl thiocyanate alone in a concentration equal to the total concentration of the mixture.

THIOCYANATES AS POSSIBLE STOMACH POISONS

While many thiocyanates have been shown to be toxic to insects when used as contact sprays, information regarding their possible value as stomach poisons appears to be lacking. Accordingly feeding experiments were performed using silkworm (*Bombyx mori* L.) larvae, on mulberry (*Morus alba* L.) leaves which had been sprayed or dusted with thiocyanates. A modification of the sandwich method of Campbell and Filmer (1) was employed. Ten partly grown larvae were used in each test, with an equal number of check larvae fed on sandwiches of unsprayed foliage. A test was not regarded as valid unless the larvae had fed. The temperature was 25° C. When tested either as dusts or sprays trimethylene thiocyanate and phenacyl thiocyanate were entirely ineffective. Thiodiphenylamine killed 9 out of 10 larvae.

Feeding tests were made with γ-thiocyanopropyl phenyl ether. In this case the leaves or petals of the host plant were sprayed with a 0.1 per cent solution in 0.5 per cent Penetrol. When the leaves were dry about 50 insects to be tested were placed on the treated foliage and allowed to feed for at least 15 hours. Fall cankerworms (*Alsophila pometaria* Harris) that fed on treated hickory (*Carya ovata* [Mill.] K. Koch) leaves showed an average mortality of 18 per cent. Flea beetles (*Epitrix cucumeris* Harris) that fed on treated potato (*Solanum tuberosum* L.) foliage showed a mortality of 10 per cent. The presence of fresh feeding punctures indicated that the beetles had fed. Rose chafer (*Macrodactylis subspinosus* Fabr.) adults appeared to be unharmed after feeding on treated rose (*Rosa* sp.) petals.

It appears then that the thiocyanates offer little promise as stomach poisons.

TOXICITY OF THIOCYANATE SPRAY RESIDUES TO GUINEA PIGS

Little is known regarding the action of thiocyanate spray residues on higher animals or their effects on human health, although sprays that contain the SCN group are now in general use.

Leonard (5) found that the subcutaneous minimum lethal dose of ethyl isothiocyanoacetate in the rat is 0.35 to 0.50 g. per kilogram of body weight as compared with 0.26 to 0.31 g. per kilogram for allyl isothiocyanate. He states that both compounds kill by respiratory paralysis, but that ethyl isothiocyanoacetate appears to possess some motor paralyzing and perhaps narcotizing action. Taubmann (6) found the mono- and dithiocyanates to be convulsants attacking the cerebral axis of the brain in frogs and rabbits. Trimethylene thiocyanate was among the most toxic, being lethal when injected subcutaneously in rabbits at a dose of 6 mg. per kilo of body weight. This compound, she reports, pro-

duced also a marked decrease in hemoglobin and erythrocytes causing clotting of the blood.

In this study preliminary tests were made on guinea pigs. Six males 24 weeks old were confined to cages made of galvanized iron and wire. Pine shavings were used as bedding. The ration consisted of treated cabbage leaves and untreated whole oats, which was offered in excess of what the animals would accept, for a period of six weeks. The thiocyanate compound to be tested was dissolved in acetone, the solution sprayed on the cabbage leaves, and the acetone allowed to evaporate before the leaves were offered as food. Sprayings were made daily so that the residue would deteriorate as little as possible before being eaten.

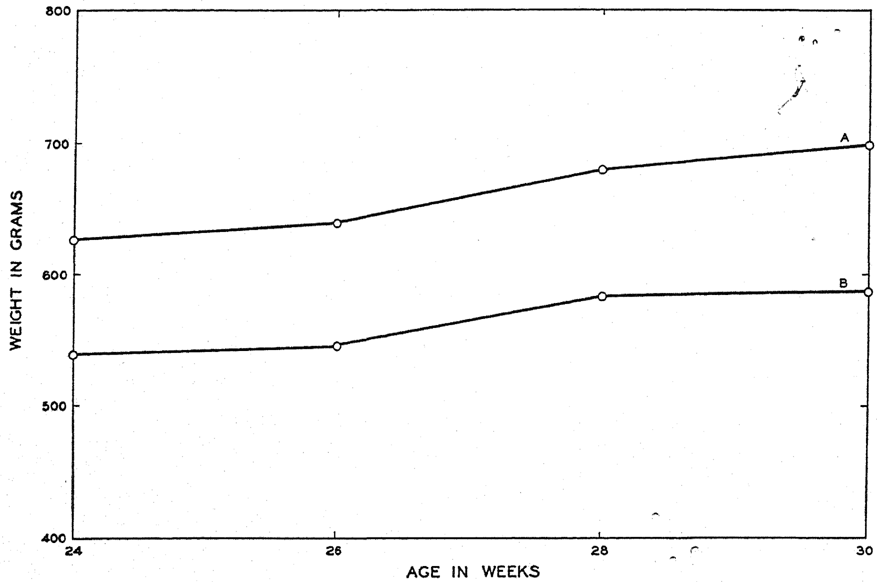

FIGURE 1. Growth curve of guinea pigs fed SCN-treated cabbage. A. Pair fed on trimethylene thiocyanate-treated cabbage. B. Control.

A pair of guinea pigs fed on 0.2 per cent trimethylene thiocyanate-treated leaves (Fig. 1 A and Fig. 2 B) showed an average increase in weight from 625.5 g. to 697.5 g. Two control animals (Fig. 1 B and Fig. 2 A) which were given a ration of untreated oats and cabbage increased in average weight during the period of the experiment from 537.5 g. to 585.5 g. The treated cabbage in all cases was readily accepted. A pair fed on leaves treated with Penetrol (0.5 per cent), the emulsifying agent of the sprays mentioned in the previous section, lost an average of 48 g. in weight. Aside from loss in weight, the individuals of this pair appeared to be normal. No irritation of the eyes or skin was noted in any of the

animals fed treated leaves. An examination of the incisors showed no appreciable injury to the enamel of the teeth. No symptoms of paralysis were noted. Both treated and the controls appeared normal in so far as neuro-muscular response could be detected by observation. As the toxic dose of the residues ingested was doubtless considerably below the lethal dose, no autopsies were performed.

An estimate of the dose based on the volume of spray solution used in producing the SCN residues on the cabbage leaves eaten indicated that it did not exceed 2 mg. per kilo of body weight per day.

Taubmann (6) found that doses of trimethylene thiocyanate less than 3 mg. per kilo injected subcutaneously in rabbits produced no noticeable effect.

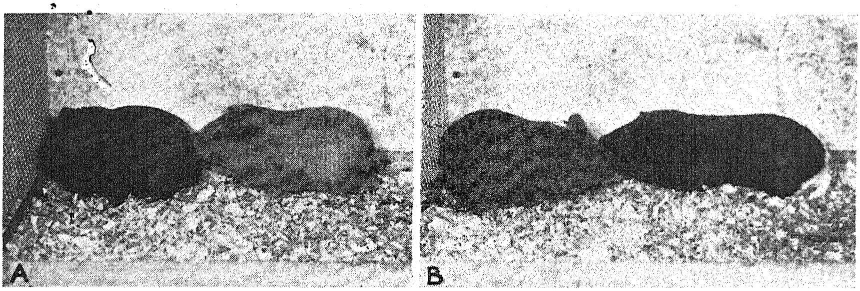

FIGURE 2. Guinea pigs used in spray residue feeding experiments photographed at the end of the six weeks' period. A. Control. B. Pair fed on trimethylene thiocyanate-treated cabbage. The individuals in this series appeared to be normal at the end of the experiment.

It is evident in the present investigation that the dose obtained by guinea pigs fed sprayed leaves was insufficient to produce characteristic thiocyanate symptoms.

SUMMARY

Trimethylene thiocyanate and lauryl thiocyanate were compared with respect to their toxicity to *Aphis rumicis*. When compared at equal concentrations of SCN, lauryl thiocyanate was found to be more toxic. This compound was, however, considerably less toxic than pure rotenone.

The toxicity of mixtures of equal parts of rotenone and lauryl thiocyanate was the same as that of a spray containing rotenone alone at a concentration equal to the total concentration of the mixture and greater than that of a spray containing thiocyanate alone at this concentration.

Trimethylene thiocyanate and phenacyl thiocyanate were ineffective as stomach poisons to silkworms.

γ-thiocyanopropyl phenyl ether was also ineffective as a stomach poison to several species of insects.

Guinea pigs fed cabbage leaves sprayed with trimethylene thiocyanate showed no acute symptoms. There was a steady increase in weight not significantly different from that of the controls.

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Summarized by ZELIAETTE TROY

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Aster Yellows and Its Control

A Brief Summary* of the Work of Dr. L. O. Kunkel in Solving An Important Growers' Problem

Aster yellows has been found in 50 different species of plants. This virus disease is transmitted by a leafhopper (Cicadula sexnotata). Aster plants grown under cheesecloth tents are protected not only from this disease-bearing insect but from many more which cause injury; the shade so furnished also improves the foliage and blossoms.

By

ZELIAETTE TROY

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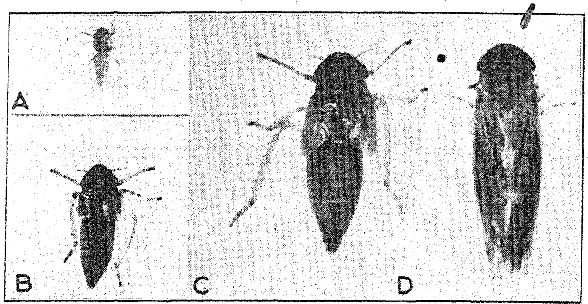
LAST year, hundreds of acres of Asters were grown under tents in the United States. The year 1935 saw many more acres under cloth, for American commercial growers have found that tents protect their Asters not only from the leafhopper which carries yellows, but from other leafhoppers, aphids, and tarnished plant bugs which cause so much damage. In addition, the shade given by these tents furnishes the right light conditions for production of long sturdy stems, firm leaves, and perfect blooms—characteristically healthy stock.

These acres and acres of cloth tents are a shining example of the application of a scientific discovery to American business. In 1923, L. O. Kunkel, then working at the Boyce Thompson Institute for Plant Research, Yonkers, N. Y. undertook the study of Aster yellows. In 1924, he announced that a leafhopper, *Cicadula sexnotata*, is probably the only insect which can transmit this disease; later investigations have confirmed this statement. At first, wire screens were used to fence in the Aster plots, for the leafhopper seldom flies higher than 6ft.; then came the idea of cheesecloth fences and finally the cheesecloth tent to keep out the wind-blown as well as the flying insects. Cloth with 22 by 22 threads per inch has proved the most satisfactory. Wooden uprights 6ft. high placed 32ft. apart in one direction and about 16ft. apart in the other with wires tightly strung over the uprights furnish the support for the cheesecloth when it is sewn together on the wires. One thrifty grower, hit by the depression, found that second-hand tobacco cloth, bought by the bale, served nicely.

THE CARRIER.—*Cicadula sexnotata* has been commonly known in all parts of the United States and Canada for 50 years; it differs little in appearance from the European form which feeds upon China Asters—but without the disastrous effects known in this country. One reason why the American species is so abundant is that it feeds and breeds on a wide range of host plants. Grasses, Oats, Wheat, Barley, Rye, Sugar Beets, Lettuce, Great Ragweed, Sow Thistle, English Plantain, Dandelions, and Moonpenny Daisy are a few of its favorite food sources as well as

*Summary based on following articles by L. O. Kunkel, who is now located at the Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.: (1) Insect transmission of Aster yellows. *Phytopath.* 14: 54. 1924; (2) Insect transmission and host range of Aster yellows. *Sci.* n. ser. 62: 524. 1925; (3) Incubation period of Aster yellows in its insect host. *Phytopath.* 126: 67. 1926; (4) Studies on Aster yellows. Contrib. Boyce Thompson Inst. 4: 181-240. 1926; (5) Studies on Aster yellows in some new host plants. Contrib. Boyce Thompson Inst. 3: 85-123. 1931. (*Available in reprint form*); (6) Celery yellows of California not identical with Aster yellows of New York. Contrib. Boyce Thompson Inst. 4: 405-414. 1932.

breeding grounds. As with most other kinds of insects, its numbers vary greatly from year to year.



Four Stages in the Development of the Carrier of Aster Yellows

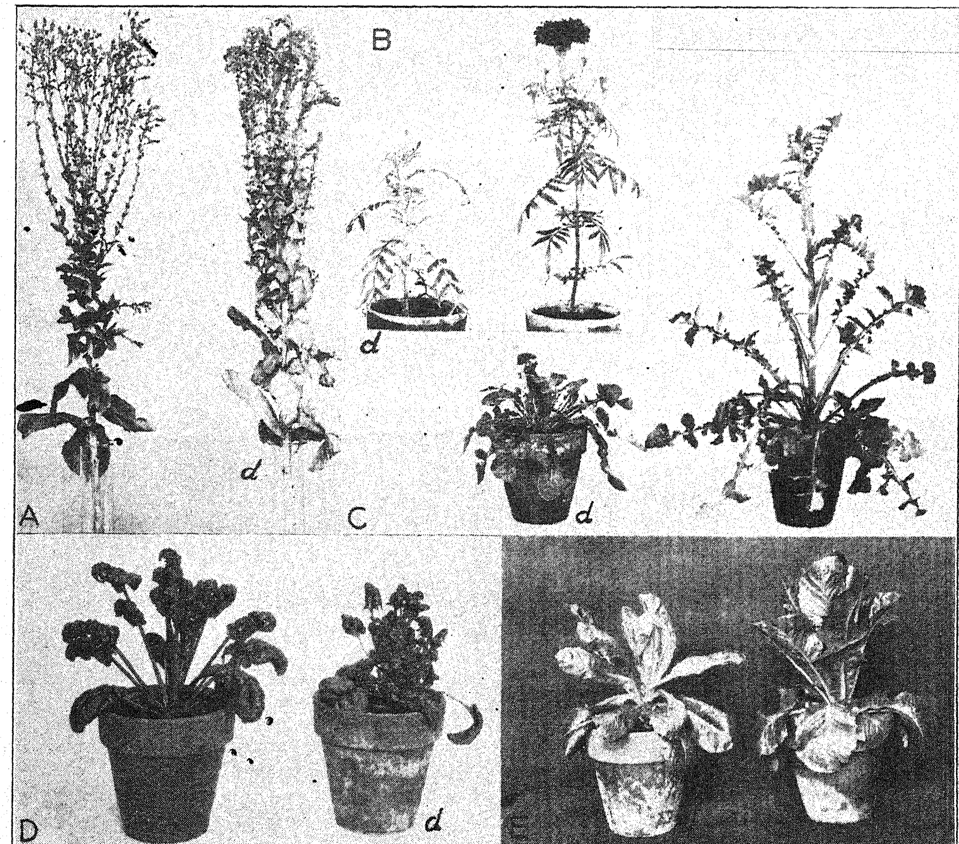
The leafhopper *Cicadula sexnotata* showing (A, B, C) three progressive nymph forms and (D) an adult. All enlarged.

When the temperature is at 75 deg. F. this insect will pass through its life cycle, from egg to egg, in about 40 days. One female, kept under careful observation during her life of 87 days, laid eggs from which 127 nymphs hatched, puncturing a hole under the upper or lower epidermis of a leaf, depositing the eggs, and then plugging up the hole with a gray waxy substance. In 11 to 13 days the eggs hatched; the adult stage was reached about 20 days later.

Winter weather kills off both adults and nymphs but not the eggs. However, Dr. Kunkel found that the virus is *not* carried through the Winter in the eggs, for newly hatched insects cannot infect a healthy plant until they have fed upon a diseased plant. Furthermore, the leafhopper cannot transmit this disease until the virus has remained in it for a period of ten days, which shows that the insect is more than a mere mechanical carrier. He also found that *Cicadula sexnotata* is the only one of the many insects which feed upon Asters which can transmit Aster yellows; Dr. Severin of California has found two additional leafhoppers which can transmit the California strain of yellows (what is known there as the Celery strain) but they cannot transmit the eastern strain.

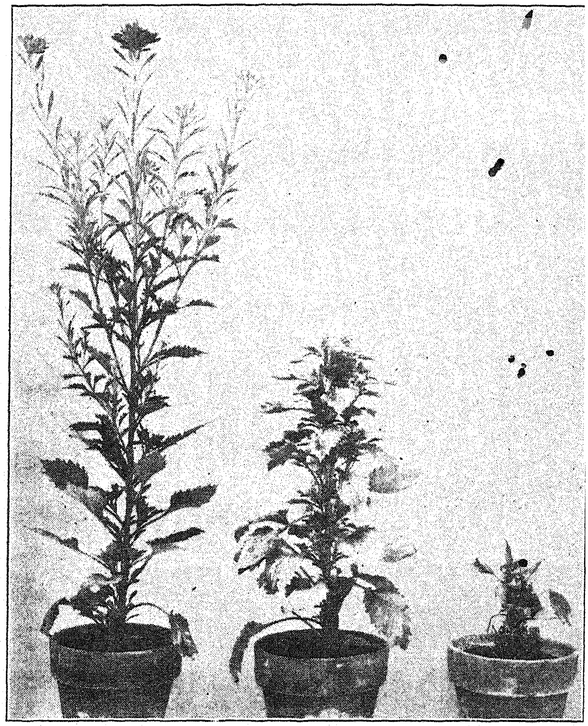
HOSTS.—As noted, the virus is not carried through the Winter in the insect egg; nor does it overwinter in Aster seeds, for seeds taken from partly diseased plants (wholly diseased ones do not set seeds) produced only healthy plants. Then where does this virus spend the Winter? Of the many plants upon which the leafhopper feeds and breeds, Dr. Kunkel found that Lettuce, Dandelions, Hogweed, Calendula, most of the Chrysanthemums, and many others showed typical Aster yellows symptoms and that if these plants were confined in cages with healthy leafhoppers and healthy Aster plants, typical Aster yellows could be produced in the Asters after ten days. In other words, a healthy leafhopper can feed upon a diseased specimen of any one of at least 50 different species belonging to 23 different families of plants and, after ten days, infect a healthy Aster plant with Aster yellows. If Winter weather killed off all diseased plants there would be no Aster yellows the next Summer. But of the 50 species which take the Aster yellows disease many are perennials or biennials; some of the most common of them belong in the genera Chrysanthemum, Sonchus, Asclepias, Erigon, and Plantago. (See list on page 266.)

SYMPTOMS.—Aster leaves affected with yellows never show mottling; therefore it is easy to distinguish Aster yellows from chloroses of the mosaic type. One of the most striking symptoms is the abnormal production of secondary shoots. These frequently arise in the axils of normally green leaves which were mature before the plant became infected and they are always thin and diseased in appearance. Plants which contract the disease before they are mature always are more or less stunted, the degree of the stunting varying with the age of the plant at the time of infection.



The Effect of the Aster Yellows Disease on Different Plant Hosts
In each pair the diseased plant is indicated by (d). A—Boston Lettuce; note chlorosis (loss of color) and upright growth. B—African Marigold; the diseased, stunted flowers are greenish. C—Sonchus (Sow Thistle); the stunted, chlorotic plant shows many upright, secondary shoots. D—Spinach; the leaves show (not in photograph) clearing of the veins. E—Romaine Lettuce; diseased plant is chlorotic and has failed to head.

The first symptom to be observed in a young plant is a slight yellowing along the veins in the whole or in a part of a single young leaf. This is known as "clearing of the veins" and, as it has not been observed in connection with any other Aster disease, it makes diagnosis possible long before conspicuous chlorosis appears. When a mature plant is attacked, the older leaves remain normal; the intermediate ones — those one-half or two-thirds grown—show clearing of veins and general chlorosis; the very young leaves are almost white but may become more or less green as they grow old. The flower petals which ordinarily contain no chlorophyll become quite green when diseased. The leaves and the individual flowers tend to stand upright instead of making a broad angle with the stem.



How Aster Yellows Affects Aster Plants of Different Ages

It is most severe on the very young ones and progressively less severe as the age increases.

CONTROL. — Asters grown in plots surrounded by cultivated fields are less subject to severe infection than those grown in the vicinity of pastures, meadows, waste lands, and other weedy places. Aster beds near buildings are somewhat less subject to disease than plantings in the open, because the Aster leafhopper is a wild insect and avoids buildings. In localities where it is impossible to remove all perennials and biennials known to be subject to the disease, the most effective means of control is the use of tents constructed of cheesecloth (22 by 22 threads per inch), and the destruction of all Aster plants as soon as they are observed to show yellows.

CONCLUSION.—Not only has Dr. Kunkel's work made possible the profitable cultivation of Asters by the florist and home gardener, but it has brought some order out of the chaos in certain phases of plant virus diseases. For he proved that Aster yellows is identical with white-heart disease of Lettuce, a previously undescribed disease of Buckwheat, and also several yellows diseases of cultivated garden plants. It is similar, but apparently distinct from, Peach yellows, Strawberry yellows, curlytop of Beets, and false blossom of the Cranberry; and it decidedly is *not* the same as the stunt disease of Dahlias. His work is also the first proof that a distinctive yellow disease is insect-transmitted.

Plants from Which Aster Yellows Has Been Transmitted to Asters by the Leafhopper, Cicadula sexnotata

Ambrosia trifida (Horseweed)
Ammobium alatum (Winged Everlasting)
Anethum graveolens (Dill)
Begonia semperflorens
Bellis perennis (English Daisy)
Brachycome iberidifolia (Swan River Daisy)
Cajophora lateritia
Calendula officinalis
Calliopsis
Callistephus chinensis (China Aster)
Centaurea margaritae
Chrysanthemum cinerariifolium (Pyrethrum)
C. frutescens (Paris Daisy)
C. leucanthemum (Ox-eye Daisy)
C. leucanthemum maximum (Moonpenny Daisy)
Chrysanthemum (Little Gem)
Clarkia elegans
Cosmos bipinnatus
Daucus carota (Carrot)
Dimorphotheca aurantiacum (African Daisy)
Erigeron annuus (Daisy Fleabane)
E. canadensis (Butterweed)
E. speciosus
Gaillardia aristata
Galinsoga parviflora
Gypsophila paniculata (Babysbreath)
Lactuca sativa (Lettuce)
Leontodon autumnalis (Fall Dandelion)
Matricaria alba (Feverfew)
Mimulus luteus (Monkeyflower)
Myosotis scorpioides (Forget-me-not)
Nemophila
Nicotiana rustica (Tobacco)
Pastinaca sativa (Parsnip)
Plantago major (Plantain)
Rudbeckia hirta (Black-eyed Susan)
Salpiglossis
Scabiosa atropurpurea
Silene pendula
Sonchus arvensis (Sow Thistle)
S. oleraceus
Taraxacum officinale (Dandelion)
Tragopogon porrifolius (Salsify)
Vinca rosea (Periwinkle)